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STUDIES IN THE INHERITANCE OF COLOR IN PERCHERON HORSES.

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I. The Influence of Selection and Inbreeding upon Prepotency.

The materials for this paper have been drawn from the records of the American and French Percheron Horse Breeding Associations. The data obtainable from breeders' books have been used extensively within recent years in the investigation of problems of heredity. The "Law of Ancestral Heredity" formulated by Galton and revised by Karl Pearson is the foremost of the deductions claimed as a result of the study of such data.

The information furnished by the Percheron register is in respect to color, sex and age, and the questions proposed in this paper involve the three sorts of data available. Three questions have been made the subject of investigation: (1) To what extent a color which has been predominant in the breed in the past shows any prepotency over a color which has been increased by breeders' selection. The case with the Percherons is that gray has been the predominant color, but black is on the increase. (2) A second question has been raised as to normal inheritance from parents of different ages, whether parents of greater age influence the offspring to a greater extent, and whether there is an optimum age of prepotency. (3) Whether either sex is prepotent over the other.

At the outset of this investigation the writer started to test the "Law of Ancestral Heredity" of Galton. To this end was collected the complete ancestry of 179 individuals up to the third ancestral generation, showing the colors of parents, grandparents

and great-grandparents. The results showed that a secular change has been in progress in the breed increasing the per cent. of black individuals. The figures are given in Table I.

The per cent. of grays among the great-grandparents was 58, and this declined to 47 per cent. among the grandparents and

TABLE I.

		G.-G.-P.	G.-P.	P.	Offspring.
White or Gray.	No. of Sires.	368	140	68	♂ 34
	No. of Dams.	458	200	73	♀ 30
	Total.	826	340	141	64
	Per Cent.	57.68	47.48	39.38	35.75
Black.	No. of Sires.	327	190	81	♂ 40
	No. of Dams.	198	140	93	♀ 51
	Total.	525	330	174	91
	Per Cent.	36.66	46.08	48.60	50.83
Bay, Brown and Chestnut.	No. of Sires.	21	28	30	♂ 9
	No. of Dams.	60	18	13	♀ 15
	Total.	81	46	43	24
	Per Cent.	5.65	6.42	12.01	13.40
	Totals.	1,432	716	358	179

to 39 per cent. among the parents. Corresponding to these there was a rise in the per cent. of blacks from 37 per cent. among the great-grandparents to 46 per cent. of the grandparents and 49 per cent. of the parents. There was also an increase in the bay-brown group from 6 per cent. to 12 per cent.

These facts correspond to the testimony of breeders in regard to the increasing popularity of black. The preference or fashion for black seems to be leading to a gradual change from the formerly predominant gray color of the breed which prevailed in its place of origin in France. The gray-white group of colors includes a number of minor varieties and is the outcome of former generations of inbreeding of animals of this color.

The 179 offspring whose ancestry has been traced are themselves divided as follows among the three color groups: Gray 64, black 91, bay-brown 24. This is a showing of 51 per cent. of blacks among the offspring, which is in excess of the per cent. found among the parents. Doubtless this is an exhibition of the breeders' preference for black which has led to an excessive registration of animals of the favorite color. The expense of

registration must be considered as a factor. Thus the 179 individuals are not in all probability a representative population, owing to the elimination of grays from the register.

It should be added that this is the surface explanation of the facts, which might be accounted for partly at least in other ways, as by an actual prepotency of the black color, or by a melanic tendency due to effect of change of climate on the breed on removal from its original home in France to America. All of the offspring and parents and almost all of the grandparents are American registered animals. It is chiefly in the great-grandparental generation that the French records have been drawn upon.

The following description of the Percheron breed is taken from a work on "The Horse" by Roberts (Macmillan, 1905): "About 1820 two noted gray Oriental stallions, Godolphin and Gallipoli, were introduced into the Government stables at Pin. These two prepotent stallions fixed the style of color and fastened it on an already susceptible breed (p. 159). . . . The color of most Percherons is gray of varied shades. Sometimes it is quite light, becoming nearly pure white in old age. Again, the striking light and dark dapples are seen, and dark grays, almost black, with a few white hairs. Comparatively few blacks have, as yet, been bred, although dark colors are sought and are more common than formerly. The American purchaser prefers darker rather than lighter colors; hence the effort in France is to produce darker colored animals than formerly. . . . It will take many generations to entirely eliminate the light colors, so long one of the characteristics of the breed; but this will be accomplished in time if Americans persist in preferring dark- rather than light-colored draft-horses. This preference is not founded on a fad, for, other things being equal, dark-colored horses are to be preferred to light-colored ones" (p. 162 ff.).

From all that we know of the history of the breed it is quite obvious that the increase in black and decrease in gray through four generations, shown in Table I., is at least partly a result of breeders' selection, and would not warrant the assumption of a melanic tendency or prepotency of black. Indeed, it is quite equally obvious that we should expect the long predominant color, gray, to be the prepotent color. Independent data have

been collected which bear upon this point, and the same data have been tabulated with reference to the two other matters mentioned at the outset, the prepotency of sex and the relation of age to prepotency.

The Selection of Data to Determine Prepotency.

With the view of determining certain of the factors of prepotency, two thousand individuals of pure black or gray color have been selected at random, consisting of one thousand colts and one thousand fillies, the parents likewise being of pure color, one black the other gray. This method of selection gives a positive resemblance of the offspring to the one or the other parent. It seemed to the writer that the pure colors were the only ones from which inferences could be drawn, since the descriptions of color are too meager to warrant a safe inference in cases of parents or offspring of mixed color. It would be impossible in the case of mixed colors to decide which was the preponderating color or to infer the resemblance of the offspring to one or the other parent. This defect is apparently inherent in data not originally collected for any scientific purpose. Any consideration of the Mendelian hypothesis is precluded. And while that fascinating theory would naturally be the first to turn one's attention to investigations of this sort, it has seemed that the limited use of data as above outlined might prove to be warrantable as a method for certain purposes proposed in this this paper. All of the data presented in this paper outside of Table I. consist of that just referred to, namely, the 2,000 individuals selected at random according to the method described, and including 1,000 colts and 1,000 fillies.

Table II.

Prepotency in Relation to Sex.

	Sire Prepotent.	Dam Prepotent.	Totals.
Colts	457	543	1,000
Fillies	463	537	1,000
Totals	920	1,080	2,000
Per Cent.	46	54	

In Table II. the data are arranged with reference to sex prepotency. It is seen that of 1,000 colts 543 or 54.3 per cent.

resemble the dam, and of 1,000 fillies 537 or 53.7 per cent. resemble the dam, or the dam is prepotent in 54 per cent. of all cases. The ratio between the prepotency of dam and sire is a little less than 5:4.

In the next table the sexes are separated on the basis of color and we find that gray dams and black sires are greatly in excess, being nearly 75 per cent. of the whole number.

TABLE III.—*Colts.*

PREPOTENCY IN RELATION TO BOTH SEX AND COLOR.

Sires.	Black.	Per Cent.	Gray.	Per Cent.	Totals.
Prepotent.	333	44.04	124	50.81	457
Non-prepotent ...	423		120		543
Totals.	756		244		1,000
Dams.					
Prepotent.	120	49.19	423	55.96	543
Non-prepotent ...	124		333		457
Totals.	244		756		1,000

TABLE IV.—*Fillies.*

Sires.	Black.	Per Cent.	Gray.	Per Cent.	Totals.
Prepotent.	322	44.98	141	49.65	463
Non-prepotent	394		143		537
Totals.	716		284		1,000
Dams.					
Prepotent.	143	50.35	394	55.02	537
Non-prepotent	141		322		463
Totals.	284		716		1,000

Taking the table for the 1,000 colts first, we find that there are 756 black sires and 244 gray sires, and correspondingly 756 gray dams and 244 black dams. The gray dams moreover are prepotent in 56 per cent. of the cases and the black dams in only 49 per cent. The gray sires are prepotent in 51 per cent. of the cases and black sires in 44 per cent. These figures do not essentially alter the previous results as to the prepotency of the dam but indicate that the gray dam is more prepotent than the black dam and the gray sire than the black sire. But the gray sire is less prepotent than the gray dam (51:56). The relations may be expressed thus: The combination of prepotent sex + sub-

potent color (B ♀) = subpotent sex + prepotent color (G ♂). For the prepotencies are nearly equal in this case (49.2:50.8).

The figures for the 1,000 fillies are nearly the same as for the colts (Table IV.). The black sires and gray dams are in excess as before, there being 716 black sires to 284 grays and correspondingly 716 gray dams to 284 blacks. The gray dams are prepotent in 55 per cent. of the cases, black dams in about 50 per cent.; gray sires in about 50 per cent., black sires 45 per cent. Here also the gray dams are more prepotent than the gray sires (55:50). Also the relation holds as before expressed. B ♀ = G ♂ (50.3:49.7).

TABLE V.

PREPOTENCY IN RELATION TO COLOR.

	Prepotent.	Per Cent.	Non-prepotent.	Per Cent.	Totals.
Gray parents.....	1,082	54.1	918	45.9	2,000
Black parents	918	45.9	1,082	54.1	2,000
Totals.....	2,000		2,000		4,000

In Table V. are condensed the data of III.-IV. arranged with reference to color alone. From this it is seen that gray parents are prepotent in 54.1 per cent. of the cases. Oddly enough this almost coincides with the prepotency of dams (Table II.) which was 54 per cent. This curious result comes from the fact pointed out above that black dams mated with gray sires are almost equal in prepotency in the case of both colts and fillies. Consequently the whole difference between the prepotency of gray and black parents arises from the unions between gray dams and black sires in which the gray dams are prepotent in 55–56 per cent. of the cases.

These results show from data independent of Table I. that black is not a prepotent color. Therefore the secular change shown in the increase of black in Table I. must be due to breeders' selection. It is also evident that breeders' preference is exercised by the more frequent use of black stallions. Although stallions are chosen for their individual superiority and prepotency it would seem that their superiority is not sufficient to make them prepotent as to color in the majority of cases. The original color of the race, gray, is prepotent, having behind it the hereditary force of previous generations of inbreeding. In the inheritance of color the tendency is to return to the original gray.

About 75 per cent. of the parents of grays are gray while only 60 per cent. of the parents of black individuals are black. This was found to be the case with the parents of 300 grays and 300 blacks selected at random from data not included in the rest of this paper. Thus the reason for the prepotency of gray is manifest from the average character of its ancestry.

The prepotency of the dam seems to be partly explained by the fact that gray dams are most numerous, but this does not wholly explain the dam's prepotency. For gray dams are prepotent in a higher degree than are gray sires (56:51 for colts; 55:50 for fillies).

It must be remembered that we are dealing with the recorded colors as yearlings and that a certain per cent. of color changes must occur later on. It would be interesting, for example, to have data bearing upon the question whether the young of both sexes tend to resemble the dam.

Pearson ('01) finds in respect to the inheritance of eye-color in man, "That the younger generation takes as a whole more after its male than its female ascendents and collaterals." In this paper the prepotency of the dam has been shown to be partly the result of association with a prepotent color, but this factor does not wholly explain the dam's prepotency although it diminishes her apparent prepotency.

The conclusion that the prepotency of the gray color is the effect of inbreeding owing to its long established existence as a racial characteristic in this breed may suggest the question as to how long would be required for the black color to become fixed by long continued selection and inbreeding. There may be some evidence as to the point in the data contained in Table XV. (see Appendix). Of the 91 black offspring 38 had both parents and at least half of the grandparents and great grandparents black. Moreover all the individuals having that amount of black in their ancestry were black. Of course this apparent result must be largely caused by breeders' selection, eliminating grays from the records. If it has any force at all as an exhibition of the effects of the cumulation of black in the near ancestry it would go to show that the selected color tends to become stamped in "indelibly" so to speak after a few generations of selective inbreeding. Any such facts, if proved, would of course

militate against Galton's or Pearson's law which ascribes a continued influence to the remote ancestry. The influence of the ancestral generations diminishes according to Galton's hypothesis in the series —.50, .25, .125, .0625, etc. According to Pearson's, a greater influence is ascribed to the remote generations, the series (numbered II.) running as follows —.50 (parental influence), .33 (grandparental influence), .22 (great-grandparental influence), .15 (great-great-grandparental influence).

II. Is There a Correlation Between Age and Prepotency?

The remaining question proposed in this paper is whether there exists any relation between age of parents and prepotency. Whether there is an optimum age of prepotency in either parent and the power of influencing the character of the offspring increases up to this point.

The data have been first arranged in two groups for the two sexes. The 1,000 colts are treated in Table VI. (*a*) and the 1,000 fillies in Table VI. (*b*).

Table VI. (*a*).

Colts.

	Sire of Same Age, or Older.	Per Cent. of Prepotency.	Sire Younger.	Per Cent. of Prepotency.	Totals.
Sire prepotent.....	265	46.50	192	44.66	457
Dam prepotent.....	305	53.50	238	55.34	543
Totals.....	570		430		1,000

Table VI. (*b*).

Fillies.

	Sire of Same Age, or Older.	Per Cent. of Prepotency.	Sire Younger.	Per Cent of Prepotency.	Totals
Sire prepotent.....	283	47.89	180	44.01	463
Dam prepotent	308	52.11	229	55.99	537
Totals.	591		409		1,000

Table VI. (*a*) shows that dams are slightly more prepotent when older, or in 55.3 per cent. of the cases against 53.5 per cent. where the sire was older or of the same age.

In the table of the fillies VI. (*b*) the dam is seen to be prepotent in 56 per cent. of the cases when older, as against 52 per cent. when younger or of the same age with the sire. These differ-

ences are slight and consistent in case of both colts and fillies in making the dam more prepotent when older and correspondingly the sire less subpotent when older.

In order to see whether there appears to be an optimum age of prepotency the data have been arranged in a number of tables that follow. In the mating of domestic animals a greater variety of crosses with regard to the respective ages of the parents is of course met with than in man. In marriage the man is ordinarily older, but among horses we have all possible combinations occurring frequently.

In arranging the data to determine if there be an optimum age the parents have been grouped as follows: I., very young, 3–4 years old. II., young, 5–7. III., medium age, 8–10. IV., older, 11–13. V., very old, 14 and over.

In Table VII. which follows are given all cases in which dams were mated with very young sires.

TABLE VII.

Sires 3 to 4 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts.....	77	106	57.92	183
Fillies.....	72	110	60.43	182
Totals.....	149	216	59.17	365

When the sire is very young 58 per cent. of the colts and 60 per cent. of the fillies resemble the dam, or the prepotency of the dam is 59 per cent. as against the average prepotency of 54 per cent. derived from Table II.

Naturally we would take next for consideration the prepotency of dams mated with very old sires, 14 and over. There were only 70 such cases in the 2,000. They show that 66 per cent. of the colts and 63 per cent. of the fillies resemble the dam, an average prepotency of the dam of 64 per cent.

TABLE VIII.

Sires of 14 Years and Older.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts.....	10	19	65.55	29
Fillies.....	15	26	63.41	41
Totals.....	25	45	64.28	70

Now leaving out very young and very old sires, the cases in which dams were mated with sires 5–13 years of age are given, first for colts and then for fillies.

TABLE IX.

COLTS. SIRES 5–13 YEARS OLD.

Age of Dam.	3–4 Years.	5–7 Years.	8–10 Years.	11–13 Years.	14 Years +.	Totals.
Sire prepotent.....	82	150	85	34	19	370
Dam prepotent.....	80	159	104	55	20	418
Prepotency of dam.	49.38%	51.45%	55.02%	61.79%	51.28%	788

When the dams are grouped as above from young to old — the prepotency of the dam runs along in a series above shown — 49, 51, 55, 62, 51, apparently showing an optimum period of prepotency somewhere in middle age. In Table X. dams mated with sires of 8–10, a more homogeneous group, are considered.

TABLE X.

COLTS. SIRES 8–10 YEARS OLD.

Age of Dam.	3–4 Years.	5–7 Years.	8–10 Years.	11–13 Years.	14 Years +.	Totals.
Sire prepotent.....	24	51	20	13	8	116
Dam prepotent.....	15	58	38	22	7	140
Prepotency of dam.	38.46%	53.21%	65.51%	62.85%	46.66%	256

Here the dam's prepotency makes a series — 38, 53, 66, 63, 47, the maximum being in middle age. In the next table the results for fillies are given.

TABLE XI.

FILLIES. SIRES 5–13 YEARS OLD.

Age of Dam.	3–4 Years.	5–7 Years.	8–10 Years.	11–13 Years.	14 Years +.	Totals.
Sire prepotent.....	80	153	95	39	9	376
Dam prepotent.....	88	142	114	40	21	401
Prepotency of dam.	52.4%	48.1%	54.54%	50.6%	70%	
	(average)			(average)		
	49.67			55.96		777

It may be noted that in the last table the prepotency of the dam does not show a regular increase nor run to so high a point at any age as in the table of colts. On the whole, however, there is a rise in the prepotency of the dam with age.

In the previous tables dams were taken of all ages. In the next are given all the cases where very young dams were mated with very young sires.

TABLE XII.

Sire and Dam 3 to 4 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts.....	20	33	62.26	53
Fillies.....	22	31	58.49	53
Totals.....	42	64	60.37	106

The dam's prepotency is high, where the ages are on an equality and just as high as in Table VII$_5$, where the dam had the supposed advantage of age.

Next is given a table showing very young dams mated with sires 5–13, the more prepotent sires.

TABLE XIII.

Dam 3 to 4, Sire 5 to 13 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts.....	82	80	49.38	162
Fillies.....	80	88	52.32	168
Totals.....	162	168	50.90	330

This table shows an equality as to prepotency between very young dams and the more prepotent sires. In Table XIV. very young dams mated with sires of all ages are included.

TABLE XIV.

Dam 3 to 4 Years.	Sire Prepotent.	Dam Prepotent.	Per Cent. of Prepotency of Dam.	Totals.
Colts.....	103	119	53.60	222
Fillies.....	105	124	54.10	229
Totals.....	208	243	53.87	451

Now let us compare the results of the last three tables, XII.–XIV., and see if there is any consistent relation between these results. Arranging them so as to show an increasing prepotency for the dam, we find that dams 3–4 years of age mated with the more prepotent sires 5–13 (Table XIII.) are least prepotent. Next comes the case of young dams mated with sires of all ages, with a prepotency of 53.8 per cent. Still more prepotent are young dams mated with their equals in age (60 per cent.)

The number of cases in which very old dams occur is too small to permit a tabulation in this manner. The results for

these and for dams of medium age can be seen by inspection of Tables IX.–XI. without any farther tabulation of the data.

We find the following idea expressed by Redfield ('03) in respect to the influence of age upon prepotency: "As between two individuals of the same breed, the same rule probably holds, that the individual which has had its characteristics more firmly fixed by inbreeding will be prepotent. In the life of an individual a character is more firmly fixed in comparative old age than in youth. Consequently we may assume in the absence of evidence to the contrary, that other things being equal, the older individual will be prepotent over the younger one." Redfield's work deals with human statistics and he aims to prove use-inheritance in the case of acquired mental powers.

It is apparent of course that color is not an acquired character except to a limited extent in regard to which the present data show nothing. It is rather obscure as to what may be meant by a character becoming more fixed by age. But that the power of a parent to influence the character of the offspring may be correlated with the time of life and consequent vigor is a matter seemingly independent of whether acquired or congenital characters alone may be transmitted. On the whole these data show that the influence of age is confined within narrow limits at least.

Conclusions.

1. There is a secular change of color in progress in the breed resulting from breeders' preference for black.

2. Gray, the long-established color, is prepotent over black.

3. The dam is prepotent over the sire in the ratio of about five to four. Gray dams are more prepotent than black dams and gray sires than black sires; also gray dams are more prepotent than gray sires and black dams than black sires. The dam's prepotency is partly due to association with the long predominant color. Gray dams and black sires are greatly in excess (nearly 75 per cent. of all).

4. There is apparently a degree of correlation between age and prepotency.

5. There appears to be an optimum age of prepotency, occurring in middle life.

In an appendix are added some further tabulations of the data of Table I., testing those results by Galton's Law.

The data given in Table I. were collected under the direction of Prof. C. B. Davenport at Chicago, with a view to testing the application of Galton's Law of Ancestral Heredity, but since the manifest action of breeders' selection has vitiated the data for deductions as to normal inheritance, for a series of generations, it is not worth while to include here any consideration of Galton's Law.

I wish to thank Mr. S. D. Thompson, Secretary of the Percheron Horse Breeders' Association, for access to unpublished records.

Appendix. The Data of Table I. in Relation to Galton's Law.

It was stated at the outset of this paper that the results of Table I. do not agree with Galton's Law of Ancestral Heredity, showing an abnormal proportion of black offspring, a fact doubtless due to breeders' selection. The excess of blacks is clearly seen by inspection without the use of Galton's method of calculation, since the per cent. of black offspring is greater than the per cent. of black parents, and the latter are greatly in excess of the number present in the two preceding generations. Galton's method of calculation was applied to these data, but it is not worth while to include those calculations here in view of the fact that the manifest action of breeders' selection has vitiated the data for deductions as to normal inheritance. The general result of the calculations may be given, however. They were made according to Galton's original method, not by Pearson's method of correlation, by which he makes use of far more data than the ancestry of 179 individuals would furnish.

The results are briefly: Out of 179 offspring the number of blacks calculated was 74, the actual number being 91. Of the grays the number calculated was 77, the actual number was 64. The bay-brown group exceeded calculations considerably, comprising the remaining 24 individuals.

At the right in Tables XV.–XVI. are given the per cents. of blacks and grays in the consecutive classes beginning with those

having the highest amount of the same color in the ancestry and running down. The series run:

Grays,	83	65	61	44	17	2	0
Blacks,	100	91	79	38	( )	14	0

Comparing these series it is seen that the top class of blacks comprises 100 per cent. of black individuals and, throughout, the series runs higher than for the grays. This may be interpreted as the result of breeders' selection either wholly or in part, a

TABLE XV.

SHOWING NUMBER OF BLACKS IN ANCESTRY OF 179 INDIVIDUALS, FOR THREE ANCESTRAL GENERATIONS.

No. of Black Parents.	No. of Black Grand-Parents.		No. of Black Great-Grandparents.										Per Cent. of Blacks.
			8	7	6	5	4	3	2	1	0	Totals	
	4	*a*		1	1	1	6	4	1		1	15	100
		b		1	1	1	6	4	1		1	15	
	3	*a*				9	1	3		1		14	
		b				9	1	3		1		14	
2	2	*a*				4	5	5	2	2		18	
		b				4	5	4	1	2		16	
	1	*a*					1		1	1		3	90.9
		b					1		1	1		3	
	0	*a*							1			1	
		b							1			1	
	4	*a*							1			1	78.9
		b							1			1	
	3	*a*				4	8	5	1			18	
		b				3	6	4	1			14	
1	2	*a*			1	7	5	3	6			22	
		b			1	3	2	1	3			10	
	1	*a*				3	1	6	3	3	2	18	38
		b				2	1	3	1	0	0	7	
	0	*a*					2		3	3	2	10	
		b					1		1	0	0	2	
	4	*a*											
		b											
	3	*a*											
		b											
0	2	*a*				2	2	2	7	7		20	
		b				1	2	1	1	1		6	13.5
	1	*a*				3	3	3	11	1	6	27	
		b				0	1	1	0	0	0	2	
	0	*a*						1	7		4	12	0
		b						0	0		0	0	
	Totals.	*a*										179	
		b										91	

NOTE.—*a* equals number in class; *b* equals number of blacks.

matter which is wholly beyond determination. It has been discussed above whether these results show the effects of inbreeding of blacks (p. 271).

Galton's law and Pearson's modification of it have both been criticised by advocates of the Mendelian hypothesis (Castle, '03). It is not included in the purpose of this paper to consider that law except in incidental connection with Tables I., XV. and XVI.

TABLE XVI.

SHOWING NUMBER OF GRAYS IN ANCESTRY OF 179 INDIVIDUALS, FOR THREE ANCESTRAL GENERATIONS.

No. of Gray Parents. I.	No of Gray G. P. II.		No. of Gray Great-Grandparents. III.										Per Cent. of Grays.
			8	7	6	5	4	3	2	1	0	Totals.	
2	4	*a*	3		4	1						8	83.3
		b	3		3	1						7	
	3	*a*	5		6	3	1		1			16	
		b	4		6	2	0		1			13	
	2	*a*		2	8	1	5		1			17	64.7
		b		2	3	1	4		1			11	
	1	*a*											
		b											
1	4	*a*		2	4	1	1					8	60.7
		b		2	4	0	0					6	
	3	*a*	5		6	5	1	3				20	
		b	4		3	3	1	0				11	
	2	*a*			8	2	3	7	3			23	43.7
		b			5	2	1	4	1			13	
	1	*a*			1	2	4	1		1		9	
		b			0	0	1	0		0		1	
0	4	*a*			1							1	16.7
		b			0							0	
	3	*a*			3		1	1				5	
		b			1		0	0				1	
	2	*a*		2	2		10	4				18	2.3
		b		0	0		1	0				1	
	1	*a*			1	2	6	14	2			25	
		b			0	0	0	0	0			0	
	0	*a*		1	1	3	7	12	3	2		29	0
		b		0	0	0	0	0	0	0		0	
	Totals.	*a*										179	
		b										64	

NOTE.—*a* equals number in class; *b* equals number of grays.

ZOÖLOGICAL LABORATORY,
NORTHWESTERN UNIVERSITY,
Evanston, Ill., June, 1905.

LITERATURE.

Blanchard, N.

'02 On Inheritance in Thoroughbred Racehorses. Biom., Vol. II., pp. 229-234.

Castle, W. E.

'03 The Laws of Heredity of Galton and Mendel, and Some Laws Governing Race Improvement by Selection. Proc. Am. Acad. of Arts and Sci., Vol. 39, No. 8.

Galton, F.

'97 The Average Contribution of Each Several Ancestor to the Total Heritage of the Offspring. Proc. Royal Soc. London, Vol. 61, pp. 401-413.

Lee, Alice.

'02 On Inheritance in Thoroughbred Racehorses. Biom., Vol. II., pp. 234-236.

Pearson, K.

'00 The Grammar of Science. London, 1900, 548 pp.

Pearson, K., and Alice Lee.

'01 Mathematical Contributions to the Theory of Evolution. Part III., On the Inheritance of Eye-Color in Man. Phil. Trans. Royal Soc. London, Series A, Vol. 195, pp. 102-118.

Pearson, K.

'02 The Law of Ancestral Heredity. Biometrika, Vol. II., pp. 210-228.

Redfield, C. L.

'03 Control of Heredity. Chicago.

Roberts, I. H.

'05 The Horse. Rural Science Series. Macmillan.

PERCHERON STUD BOOKS.

'88 and '98 Percheron Stud Book of America. Pub. by American Percheron Horse Breeders' Association.

'83, '85, '87 and ff. Stud Book Percheron de France Autorisée par le Gouvernement.

SECTIONING PARAFFINE AT A TEMPERATURE OF 25° FAHRENHEIT.

KATHARINE FOOT AND E. C. STROBELL.

It is with some hesitation that we publish our troublesome device for cutting thin sections of the egg of *Allolobophora*, for in other material investigators are able to obtain thin sections without recourse to such complicated methods.

Our method is quite opposed to established rules, for the best authorities on technique recommend sectioning in a temperature varying, according to the season, from 60° to 70° Fahrenheit, in some cases employing heat reflected from a lamp, and they advise the use of paraffine having a melting point not higher than 45° to 50° C., emphasizing the fact that harder paraffine is "most hurtful to tissue." [1]

We found it necessary, however, to use paraffine of a much higher melting point, working slowly toward the use of this harder paraffine, and comparing the results step by step, in order to demonstrate whether the hard paraffine was really harmful to the egg structures. As no injury to the cytoplasmic or nuclear structures could be detected, we finally imbedded the eggs in the hardest paraffine obtainable, that having the melting point registered at 74° C. With this paraffine, a Thoma microtome, and the knife in the best possible condition, we were able to get moderately good sections of 5 and 6 μ, but an attempt to cut thinner sections crushed the paraffine enough to destroy the spherical form of the egg. What was gained by the thinner sections being more than sacrificed by the distortion of nearly all the constituents of the egg.

We tried to increase the hardness of the paraffine by devising an object carrier that would hold a piece of ice or iced water, but there was little gained by this method. The paraffine could be safely cooled only to a very limited degree below the temperature of the room, beyond this, moisture formed on the block and

[1] Bolles Lee, "The Microtomist's Vade-Mecum." 1896.

serial sections were impossible. We also tried Bütschli's method of painting each section with a thin layer of colloiden. In this way Bütschli obtained satisfactory sections less than 1 μ thick, but we failed to get like results.

We were convinced that the difficulties were not due to mechanical defects in the microtomes, for we were able to cut sections 3, 2 and even 1 μ with almost every microtome we have tried. But in these thin sections, the perfect contour of the eggs was destroyed, their diameter in some cases being reduced more than one half. This we demonstrated by cutting a series of sections, the first half dozen 10 μ, the next half dozen 5 μ, then 4, 3, 2 and 1 μ. Comparing the last sections of the series with the 10 μ section showed how much the structure of the egg was sacrificed to the thin sections, and convinced us of the necessity of devising some special method to harden the paraffine in order to secure thin sections in every way up to the standard of the 10 μ section.

Experimenting with a block of pure paraffine less than one eighth of an inch square (without any imbedded object) and setting the microtome at 3 μ, we gradually lowered the surrounding temperature until each section of the paraffine maintained the exact size of the original block.

The text-figure on page 283 illustrates the freezer we finally designed to enable us to cut serial sections of 1 ½ to 2 ½ μ at a temperature about 25° Fahrenheit. The work table, on which the freezer is operated, is placed close to a north window, and on the table we put a heavy cotton pad, covering this with a heavy rubber sheet. Then the microtome (Thoma in our case) is set in place on the rubber sheet. Near the microtome we arrange the wooden object carriers, each with its subject ready for final cutting; the necessary number of clean slides and boxes (with close covers) just large enough to hold a slide.[1] Then the freezer is put in place over the microtome, the work table forming the bottom of the operating compartment *D*. The ice chamber *B* is then packed with alternate layers of cracked ice and salt, the corks tied securely in the hand holes *H*, and a rubber tube fitted over the drain tube *M* with the free end in a pail under the

[1] We use for this purpose the shallow tin boxes formerly made for typewriter ribbons.

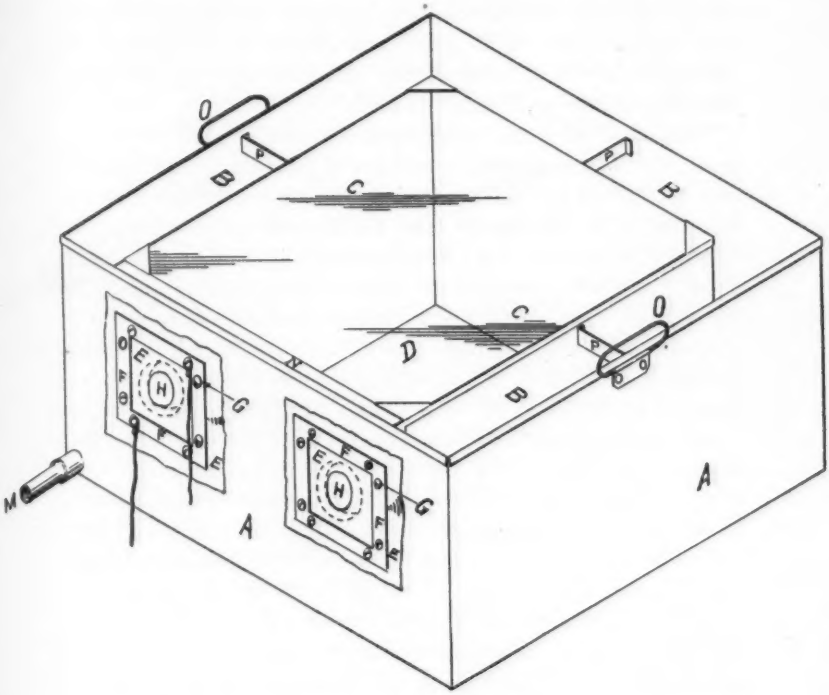

FIG. 1. The above figure is drawn to scale one eighth full size. *A*, exterior of copper box. *B*, ice chamber. *C*, plate glass ¾ inch thick, forming the top of operating compartment *D*. *D*, operating compartment The work table on which the microtome is placed forms the bottom of the operating compartment, which has the same area as the plate glass top, *C*. A thermometer is suspended in the compartment just above the microtome. This is done by glueing two loops of tape to the plate glass cover *C* and slipping the thermometer through these loops. We sometimes place a second thermometer on the bottom of the operating compartment, but the temperature registered by the one near the knife is the proper guide. *E*, heavy sheet rubber, which is kept in place by the copper frame, *F*, and copper bolts, *G*. *F*, copper frame. *G*, copper bolts which pass through the frame *F*, sheet rubber, *E*, and air chamber, *N*. The free ends of these bolts with nuts are in the operating compartment, *D*. *H*, hand holes in the heavy sheet rubber. The outer broken circle indicates the larger holes through the copper air chamber, *N*, and these must be large enough to admit the forearm freely. The hand hole, *H*, in the sheet rubber, must, on the contrary, be small enough to clasp the wrist tightly, to prevent escape of cold air from the operating compartment *D*. The sheet rubber must be renewed at least once a year, or as often as it becomes hard and loses its elasticity. When not operating the microtome, the hand holes, *H*, must be tightly closed with

table. After this the freezer must be covered with a heavy quilted covering and allowed to stand undisturbed for several hours. As a rule we "set" the freezer at night, and the following morning sectioning can begin at once.

When sectioning it is necessary to wear heavy gloves covering not only the hands, but the wrists and forearm. This obviates the discomfort of cold hands and prevents a too rapid rise of temperature in the freezer from the warmth radiating from the hands. To prevent frost forming on the glass cover, we have found it advisable to select cold days for sectioning, and to keep the window open and the temperature in the room below 50° Fahrenheit. The clear plate glass cover admits all the light needed for sectioning, but in setting the block a clearer view is obtained by reflecting the image from a small magnifying mirror.

As soon as a block is sectioned, the paraffine ribbon is lifted with a camel's hair brush to one of the cold slides in the freezer, the slide then carefully placed in one of the tin boxes and tightly covered. This is taken out of the freezer through the hand hole *H* and can be put in a cool place, fixing on the slide with the warm water method being deferred until all the blocks are sectioned.

After each block is cut, the next is immediately set for cutting, and the freezer again covered for ten or fifteen minutes. For during the process of sectioning the temperature in the operating compartment rises a few degrees, and the freezer must be kept covered until it has dropped again to 25° F. If while sectioning the knife becomes moist, the hands must be taken out at once, the hand-holes closed with the corks, and the freezer allowed to stand until the proper conditions of temperature are restored. Even the thinnest and longest ribbons do not snap or curl if the temperature in the operating compartment is not allowed to rise above the temperature of the knife.

In this way we cut from twenty-five to fifty blocks a day, all the material collected and imbedded during the summer requir-

large corks, held in place with two narrow tapes (shown in left opening). One of these tapes is passed through the ring of a screw in the center of the cork, and the tapes securely tied. If this is neglected the cold air in the freezer will force out the corks and cause a rapid rise of temperature in the operating compartment. *M*, drain tube fromi ce chamber. *N*, narrow air chamber, which is filled with cotton or some other non-conductor. *O*, copper handles. *P*, copper braces in ice chamber.

ing only a few days' work to prepare for study as needed. We always use very small blocks with four to six eggs arranged in a row in each block, and never more than one pair of ovaries in one block, as we find difficulties multiply with the increased size of the blocks, sections from the smaller blocks showing less tendency to curl or break.

It might seem that placing the microtome near an open window on a cold day would accomplish all we claim for the freezer, but the draught from an open window is sure to break the paraffine ribbon. Even in a cold room with closed windows, it is quite impossible to exclude currents of air, and the warm breath of the operator close to the microtome is a dangerous factor. In the freezer, the operating compartment is so nearly air tight that air currents are practically excluded. When the weather is intensely cold, we have sometimes used the freezer without ice, merely as a cover for the microtome, to enable us to section close to an open window, but we have always obtained the best results by using ice, and an even temperature as near 25° F. as possible.

For several years we have used this method of cutting thin sections of the egg of *Allolobophora*, for we had found it impossible to secure satisfactory thin sections of this egg with the methods in common use. Other investigators, however, rarely complain of difficulty in obtaining thin sections, and this may be due to fixing and hardening their material to a degree that makes any further hardening of the imbedded mass unnecessary, or to the fact that a large number of cells imbedded in one block of itself presents a harder mass than the single row of eggs we imbed in each block.

In our material these thin sections have been of the greatest value in aiding the interpretation of several obscure points; the constancy of the centrosome, for instance, was not demonstrated for this egg until we had secured sections of 3 μ or less. And for photographic reproduction at high magnification, thin sections offer very decided advantages, 3 μ representing the maximum thickness we have been able to use most successfully. It is possible of course to photograph the different planes of a thick[1] as

[1] With our method of focussing, a thick section requires a focussing (minus spherical) lens of lower power than the one used for thin sections.

well as a thin section, selecting the detail needed in each plane, but the structures above and below the selected plane being out of focus produce in thick sections a badly blurred image unfit for the best photographic reproduction.

WOODS HOLE, MASS.

HERMAPHRODITISM IN SABELLA MICROPHTHALAMA VERRILL.

LOUISE HOYT GREGORY.

In *Sabella microphthalama* Verrill, a paired, hermaphroditic sex organ appears in each segment of the body posterior to the pharynx. The study of this hermaphroditic condition was suggested by Professor Treadwell, who collected the material at Woods Hole, Massachusetts, in 1902–1903. The specimens were preserved in Hermann's fluid and stained in iron hæmatoxylin.

In general, the arrangement of organs as seen in cross section

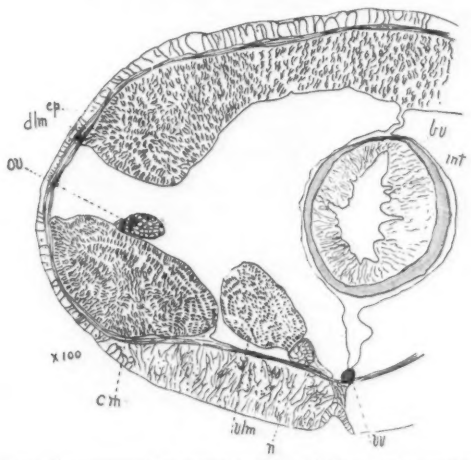

FIG. 1. Semi diagrammatic cross section through the anterior portion of the body; *bv*, blood vessel; *cm*, circular muscles; *dlm*, dorsal longitudinal muscles; *ep*, epidermis; *int*, intestine; *n*, nerve cord; *ov*, ovary; *vlm*, ventral longitudinal muscle; *vv*, ventral blood vessel.

through the body is similar to that of other annelids. The paired sex organ is found on the dorsal side of the ventro-lateral bands of longitudinal muscles in the anterior end of each segment posterior to the setæ. It is supplied with blood from branches of the ventral blood vessel. Fig. 1 is a semi-diagrammatic draw-

ing of one half of a cross section through a segment toward the anterior end of the body, showing the position of the sex organ in its relation to its surroundings.

Animals killed during the months of April, May, June, July and August differed from one another with respect to the form and contents of the sex organ, as well as to the condition of the body cavity.

All specimens killed in April and the early part of May were found to be pure females. Fig. 1 is a cross section through the body of a specimen killed April 27, showing the organ, which is a pure ovary. Fig. 2 is a magnified drawing of the ovary in Fig. 1. This is a typical organ found during the early months. It is a long, somewhat pear-shaped body, one end extending into the body cavity, the other end being attached by a band of tissue to the lateral muscles. At this same end is found the blood vessel. The cells are irregularly arranged and are in different stages of development; the majority of them contain very large nuclei with one or more nucleoli. In this organ there seems to be no definite arrangement of cells, large and small being intermingled. In the early part of April the organ is much smaller but it has the same characteristics as the one described. In none of these animals are the sex products found free in the body cavity.

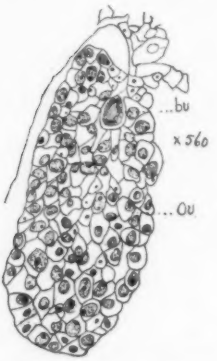

FIG. 2. Magnified drawing of ovary in Fig. 1. *bv*, blood vessel; *ov*, ova.

The animals killed during the months of May, June and July, with one exception, were hermaphrodites. During these months large masses of spermatozoa, and ova varying from the small oögonium to the large ovarian egg, were found free in the body cavity. Fig. 3 is a section through the body cavity of a specimen killed July 13, showing the different stages in the development of the ova as well as the masses of spermatozoa, both found free from the organ. *A* and *B* are sections through the outer surface of two large ovarian eggs. In the next section they have an appearance similar to that of *C*, *D* and *E*.

During August both hermaphrodites and females were found, the former being the more common.

Of the entire number examined, one third were distinctly female, and all but three of this number appeared among the specimens killed during April. No pure males were found in the material at my disposal. From these observations, it appears that the sexes are distinct at the beginning and end of the season, while during the middle of the season the sexes are united.

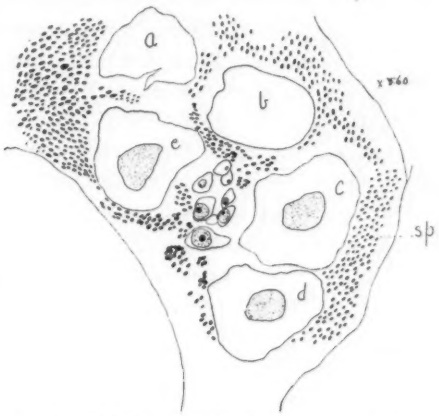

FIG. 3. Section through body cavity showing free ova and spermatozoa. *a*, *b*, *c*, *d*, ovarian eggs; *sp*, spermatozoa.

A typical hermaphroditic organ is seen in Fig. 4. It differs from the typical ovary in that it is shorter, broader, and more kidney shaped. Although ova and spermatazoa are found scattered throughout the organ, the majority of the ova appear massed together at the broader anterior end, while the larger number of male cells are found at the opposite end of the organ near the blood vessel. The ova are all in about the same stage of development. The cells on the very outer edge are ready to fall into the body cavity, where they undergo their further development. The male cells are in different stages of karyokinesis. In many cells the chromatin appears as a large, deeply stained mass, in some spindle shaped, in others in the form of an aster. In a few cells spermatozoa are found almost mature. Outside of the organ in

the body cavity are seen cells still undeveloped, cells almost mature with a thin membrane about them, and cells entirely free. Together with these are found the ova. As a general rule the spermatozoa seem to pass through the greater part of their development in the organ before passing into the body cavity, while the ova fall into the body cavity in an immature condition and undergo the greater part of their development free from the organ.

In a great many cases, spermatozoa could not be found in the organ but were found in great masses in every segment. Ova filled the organ and were also free in the body. In other specimens spermatozoa were found in the posterior end of the body cavity only, while ova were found in the organ and free in the body cavity. From these observations it appears that the male products develop first in the organ, the development beginning at the anterior end of the body. In the cases where spermatozoa were found only in the body cavity, they had already developed in the organ and had become freed. In the cases where they were found only at the posterior end of the body, those at the anterior end had become mature and had passed entirely out of the body.

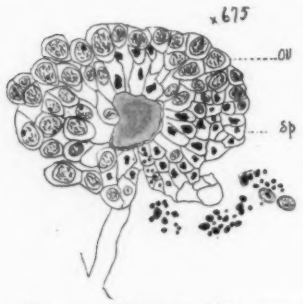

FIG. 4. Hermaphroditic organ. *Ov*, ova; *sp*, spermatozoa.

A comparison of these results with those found in *Ophryotrocha puerilis*[1] by Eugen Korschelt, shows many similarities as well as differences. In both annelids ova and sperm are found together in the same segment. In *Ophryotrocha* the sexual products are produced in a gland which has no definite structure, and which is merely a large sac in the body cavity. In *Sabella* the sex organ is more definite in outline and form. In this organ the sexual products are formed and develop until they pass into the body cavity where their appearance is similar to the appearance of the sexual gland in *Ophryotrocha*. In *Ophryotrocha* the sexual glands are found in about ten segments of the body. In the

[1] Eugen Korschelt, "Ueber Ophryotrocha puerilis Clap. Metschn.," *Zeit. f. Wiss. Zool.*, 1893-94, Bd. 57, pp. 272.

anterior segments, the glands have male characteristics, in the central segments hermaphroditic characteristics, and in the posterior segments the glands appear to be pure ovaries. In *Sabella* the ova and spermatozoa are found together throughout the organ in every segment of the body posterior to the pharynx. In *Sabella*, no cells were found corresponding to the nurse cells of *Ophryotrocha*. They evidently do not appear as ova were observed in many stages of development. In *Ophryotrocha* pure males and females were found besides hermaphrodites having male characteristics yet containing ova, and those having female characteristics yet containing spermatozoa. *Sabella* shows only two conditions, that of a pure female and that of an hermaphrodite having both female and male characteristics. The specimens appearing to be pure females could not have been hermaphrodites with the spermatozoa already developed for in the case of the April 1st forms, the organ as well as the whole animal was too small to have already produced spermatozoa. Where the ovaries were large no spermatozoa or trace of them was found in the body cavity as would have been expected if they had already developed.

Somewhat similar conditions of hermaphroditism have been observed in *Hesione sicula*[1] by W. Bergemann. Here a long gland is found differing in shape from the sex organ of *Sabella* but producing both ova and spermatozoa. This gland is seen only between the sixth and sixteenth segments. It is not found throughout the body as in the case of *Sabella*. Nurse cells are also seen in connection with the ova which is unlike the condition of *Sabella*. Like *Hesione*, the male cells in the hermaphroditic organ of *Sabella* are generally nearer to the blood vessel as is seen in Fig. 4, and usually the more mature the sex products are, the nearer are they to the outside of the organ. Whether or not the organ is developed from the walls of the blood vessel as in the case of *Hesione*, could not be determined from the material at my disposal.

VASSAR COLLEGE,
May 30, 1905.

[1] W. Bergemann, "Unterschungen uber die Eibildung bei Anneliden," *Zeit. f. Wiss. Zool.*, 1902, Bd. 73. "Ueber das spatere Schicksal der Zwitterdrusen von Hesione sicula," Zool. Anz., 1902–3, Bd. 26.

THE OSTEOLOGY OF CAULARCHUS MÆANDRICUS (GIRARD).

EDWIN CHAPIN STARKS.

The family Gobieosocidæ to which *Caularchus* belongs was at one time associated with the family Liparidæ on account of a ventral sucking disk possessed by both of them. Dr. Gunther[1] in 1861 worked out the anatomy of the disks, and showed that they were very different in structure. There being no other similarities between these two families the Gobieosocidæ has since that time been variously placed in the suborder Acanthopteri by different authors, as near the Blenniidæ, the Gobiidæ or the Batrachoididæ. Apparently the most rational disposition of the family has been made by Dr. Gill, who created for it the order Xenopterygii, which he placed with other orders of doubtful relationship with which it had nothing in common.

In undertaking the following work my interest was (1) to work over and describe the many osteological peculiarities of *Caularchus*, and (2) to attempt to find some indication of its relationship and systematic position. In the latter I have had small success, and I present this paper with reference to what anatomical value it may have.

The form I have chosen is *Caularchus mæandricus*, a species taken in abundance on the California coast. I have also examined *Gobiesox sanguineus* from the coast of Peru, and have added notes on its characters where they differ from those of *Caularchus*.

Cranium.

The cranium is much compressed and as wide as it is long. The vomerine region is broadly notched in front. On the superior surface and extending back to between the posterior margins of the orbits is a broad depression for the reception of the flat premaxillary processes. There is no supraoccipital crest and only very low temporal crests, which are connected across the

[1] Catalogue of Fishes of the British Museum, III., p. 495.

parietal region by a low transverse ridge. Long lateral processes from the prefrontal and sphenotic bound the orbital region. Broad wings from the sides of the parasphenoid form a wide floor anterior to, and in continuation with, the floor of the brain-case. The basisphenoid and opisthotic are absent. There is no myodome. In *Gobiesox* the anterior portion of the cranium is scarcely depressed for the reception of the premaxillary processes.

The basioccipital is a flat bone not turned up at its lateral edges, and the greater part of it is covered by the wide parasphenoid. Its condyle is depressed, ellipitical in shape, and slightly inclined upward in opposition to the exoccipital condyles which are slightly inclined downward.

The exoccipitals are widely separated by the interposition of the basioccipital between them. They assist the latter bone in connecting the cranium with the vertebral column, presenting a round condyle at each side of the basioccipital condyle, so that the three parts of the basioccipital condyle are on a horizontal line.[1]

The surface of the supraoccipital is divided into two parts by the meeting of the parietals over its middle. The visible anterior portion of the supraoccipital thus separated from the posterior portion is nearly round in outline. In a few of the specimens examined the parietals just touch, in none of them do they meet broadly. In *Gobiesox* the parietals are well separated by the supraoccipital, extending over the edge of the latter only slightly.[2]

[1] The condyles of the exoccipitals are wholly lateral to that of the basioccipital only in those fishes with a much depressed form, as *Callionymus*, *Remora* or *Caularchus*, though it does not at all follow that all fishes with depressed forms have lateral occipital condyles. It appears to be the rule that fishes with a depressed form never have the condyles of the exoccipitals wholly superior and in contact with each other, while those with a compressed form never have them wholly lateral.

[2] The condition of the meeting of the parietals should be more fully reported upon in other forms. Their union or separation by the supraoccipital has been used in the past in distinguishing large groups, and though the character has doubtless much less value than has been ascribed to it, its real value can not be known until it is more fully investigated. The difference between the condition of the parietals in *Caularchus* and *Gobiesox* is but a difference in degree where in one form they develop over the supraoccipital a little further than in the other and meet. This condition obviously has not the importance it has in some of the cyprinoid fishes where the parietals meet broadly entirely in front of the supraoccipital, and are the roof bones of the cranium in this region. The brief statement "parietals meeting" or "parietals separated by the supraoccipital" is far from being adequate. The condition of the parietals in *Caularchus* is not a very unusual one.

There is a notch on the posterior lateral surface of the epiotic formed between a short process above and the projecting lower edge of the epiotic below, into which the head of the posttemporal is received.

A long process projecting from the lateral edge of the pterotic passes along the posterior edge of the hyomandibular, and reaching almost to the opercle rigidly holds the suspensorium obliquely outward. A similar process formed by the sphenotic and the posterior end of the frontal projects over the anterior edge of the hyomandibular head, and forms the posterior orbital margin.

The frontals are broad and their anterior part as far back as the posterior margin of the eyes bears a large square depressed area in which the long flat premaxillary processes play.

The parasphenoid reaches its greatest width at its extreme anterior end where two wings reach far out on the prefrontals. It grows narrower below the orbital region, but again broadens in front of the proōtics, and thence tapers quickly to a point at the posterior end.

The opposing proōtics nearly meet at the median line above the parasphenoid, and in front of the basioccipital. The main parts of the fifth and seventh nerves occupy a notch in the anterior part of the proōtic, but a small branch of the former runs through a small foramen just behind the notch.

The prefrontal is a wide bone projecting laterally in front of the orbital cavity, and supported behind by the broad parasphenoid. It is pierced near its center by the olfactory nerve.

The ethmoid is disk-shaped and apparently wholly membranous in origin. It is situated between the anterior ends of the frontals and does not extend in front of them.

The vomer is very broad, having a broad shallow notch in its anterior end, and with its lateral angles projecting. In a notch formed between each lateral angle of the vomer and the end of the palatine the maxillary fits just above its middle.

The Lateral Bones of the Head.

The hyomandibular, where it articulates with the cranium, terminates in two knobs, which fit into concavities in the sphenotic and pterotic. Below it has three articular processes; one for the

opercle, one for the preopercle (by far the largest), and one divided between the symplectic and interhyal.

The symplectic is a slender bone bridging a large open space in front of the preopercle, and is the only bone between the hyomandibular and the quadrate in this region. It is received in a deep wedge-shaped notch, cut entirely to its tip, in the quadrate. The mesopterygoid and metapterygoid being absent the symplectic forms part of the anterior border of the cheek bones. In *Gobiesox* the symplectic runs behind the quadrate as usual, cutting from the latter only a small notch.

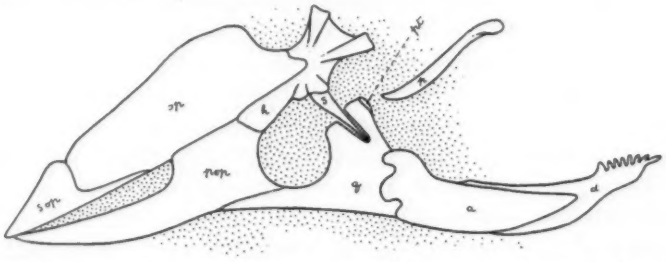

FIG. 1. Lateral bones of skull. *a*, articular; *d*, dentary; *h*, hyomandibular; *op*, opercle; *p*, palatine; *pt*, pterygoid; *pop*, preopercle; *q*, quadrate; *s*, symplectic; *sop*, subopercle.

The quadrate extends back covering the front of the preopercle. Above it is a very large open space, nearly circular in outline, bounded behind by the preopercle, and above and before by the hyomandibular and symplectic.

A small bone closely attached to the upper anterior edge of the quadrate is all that remains of the pterygoid. To it the palatine is attached by a ligament. It is very inconspicuous, and so closely united to the quadrate that it appears as a part of that element, but is easily separated in a macerated specimen.

The palatine is free from the other cheek bones, and is only connected to the reduced pterygoid by a ligament. Anteriorly it hooks over the maxillary.

The mesopterygoid and metapterygoid are entirely absent.

The articular is hinged to the quadrate by a very wide horizontal joint, making the jaw very rigid laterally. At the upper posterior angle is a strong process. There is no open space between the articular and the upper limb of the dentary.

The dentary is a strong bone and so closely attached to the articular that there is no play between them as is usual in other forms.

The angular is a very small nodule of bone on the inner surface of the articular, entirely hidden when the skull is viewed laterally. The interopercle is attached to the angular by a ligament.

The opercle, preopercle and subopercle together form an isosceles triangle with its greatest length extending backwards. The subopercle forms the posterior point of the triangle, and is interposed between the points of the opercle and preopercle. The preopercle is developed backward from its articulation with the hyomandibular to a long point, forming nearly the entire lower border of the opercular system. It forces itself between and entirely separates the subopercle and interopercle, bringing the opercle entirely above it. Its anterior end extends forward in a long point behind the quadrate nearly to the mandibular condyle. The interopercle is a small triangular bone, lying free behind the quadrate and hidden from sight by that bone when the skull is viewed laterally. It is unconnected except by a ligament to the angular. There is a low ridge running along the lower edge of the opercle, and a very high sharp one running along the lower edge of the preopercle and the quadrate nearly to the condyle of the mandible.

A broad membranous preorbital plate is all that remains of the suborbital chain of membrane bones. It bears a sensory tube which opens anteriorly to the exterior through a small pore a couple of millimeters in front of the anterior nostril. The tube crosses the plate obliquely downward and backward, forking widely in front of the eye, and opening to the exterior at the lower edge of the plate through two widely separated pores. These pores appear in the skin at the upper border of the mouth 6 or 7 mm. apart.[1] A third pore 4 mm. anterior to these, and near the front of the mouth, forms the exit of the sensory tube traversing the nasal plate.[2] At the place the preorbital tube forks the plate is slightly ossified.

[1] The specimen described is 1 cm. in length.

[2] It appears probable that the suborbital sensory tube is never present in the Teleosts unossified. There may be a series of pores or nerve hillocks, as in *Porichthys*, but they do not open into a continuous tube. I have examined a large number of forms known to have no suborbital bones, but find no tube present in any of them.

The maxillary elements are very strong. The premaxillaries send back long wide processes over the top of the head to between the eyes. Each maxillary bears a notch on its upper surface for the reception of the edge of the premaxillary process. The notch is some distance from the head of the maxillary and allows it to meet its opposite fellow below the premaxillary processes, but viewed from above the maxillaries appear to be widely separated. There is a depression in the upper surface of each maxillary into which the palatine fits.

The nasals are wide bones meeting on the median line, covering the anterior part of the premaxillary processes, and interposed between the maxillaries above.

The Branchial and Hyoid Bones.

The basibranchials are entirely absent, and the hypobranchials are but slightly separated at the median line. The latter are all of the same length, very long and much constricted at the middle. They appear to be entirely cartilaginous in a fresh skeleton, but as they dry a fine hard calcareous matter becomes evident. The hypobranchial of the fourth arch is missing, as in all other teleosts (so far as known). The ceratobranchial of the fourth arch is as long as the combined length of the ceratobranchial and the hypobranchial of the third arch,[1] and opposite the union of these two elements it is angulated, so that its lower part has the appearance of being the fourth hypobranchial. The superior pharyngeals of each side are anchylosed into a single tooth-bearing bone. The inferior pharyngeals are in contact only at their anterior ends.

A very small nodule of bone between the hypohyals on the lower surface represents the urohyal, and another on the upper surface the glossohyal. Only one of the hypohyal elements is present on each side. The suture between the hypohyal and the ceratohyal runs vertically upward from the lower side of the arch half way across the arch, then turns squarely and runs horizontally backward for nearly half the length of the ceratohyal, where

[1] I am not aware of any similar arrangement in any other Teleost. The hypobrachials usually decrease in length so rapidly posteriorly that their absence on the fourth arch does not require a lengthening of the fourth ceratobranchial; all of the ceratobranchials being of about the same length.

it again turns upward and reaches the upper side of the arch. The other hyoid elements are normal. Six branchiostegal rays are present; two are attached to the outer surface of the epihyal, two to the outer surface of the ceratohyal, and two to the inner surface of the ceratohyal. In *Gobiesox* the urohyal and glossohyal are better developed, the former being scarcely reduced.

The Shoulder and Pelvic Girdles.

The posttemporal is a long simple ray of bone inclined obliquely outward and forward from its attachment with the cranium, so that its outer end is farther forward than its inner. It thus forms a sharp angle with the supraclavicle, which is inclined forward as usual. In place of the usual lower limb a long liga-

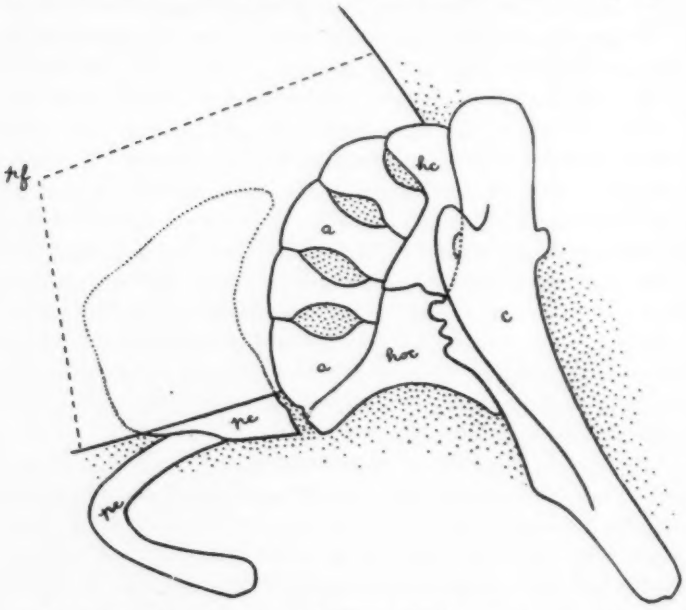

Fig. 2. Shoulder girdle. *a*, actinosts; *c*, clavicle; *hc*, hypercoracoid; *hoc*, hypocoracoid; *pf*, pectoral fin; *pc*, post clavicle.

ment connects it with the inner edge of the exoccipital near the basioccipital. Its proximal end is not very firmly attached to the cranium. A short epiotic process projects over its head, and

two stout ligaments are attached to it, one above and one below, holding it firmly against the epiotic, but leaving it movable laterally.

The supraclavicle is a long simple bone. At its lower end is a concavity which fits over a knob on the clavicle.

The clavicle is slightly bent at an angle near its middle. From its anterior edge a large lateral wing projects outward and backward. A short distance above its middle on its anterior edge is a tubercle of bone over which the cup-shaped end of the supraclavicle is attached.

The hypercoracoid is constricted at its middle opposite the base of the upper actinost so that it appears as a fifth actinost.[1] This illusion is enhanced by the pectoral fin being joined to it by two or three rays. It lies along the entire length of the upper actinost, but is separated from it, except at each end, by an open space similar to that between the actinosts. As the clavicle overlaps the hypercoracoid to the edge of the hypercoracoid foramen the foramen appears to be between these two bones. It is, however, through the middle of the hypercoracoid.

The hypocoracoid is joined as usual to the clavicle and hypercoracoid but its lower end instead of returning to the lower end of the clavicle, as usual in most fishes, projects backward along the lower edge of the lowest actinost. This condition is evidently brought about by the position of the pelvic girdle which is closely attached along the inner lower edge of the clavicle.

The actinosts are hour-glass-shaped, four in number, and separated from each other by open spaces. The coracoid elements each support two actinosts.

The postclavicle has lost its attachment with the clavicle and lies free opposite the base of the pectoral fin just behind the pelvic girdle. It is divided into two parts. The inferior portion is curved around the outer and posterior portions of the ventral disk, and the posterior fringe of the disk is attached to its edge. The superior portion is very broad and from its posterior edge is developed the peculiar fin-like flap seen externally on the fish just behind the pectoral fin. In *Gobiesox* a flat process is devel-

[1] In the accompanying drawing this loses its deceptive appearance on account of the exaggeration of the distinctness of the sutures.

oped forward from the upper element of the postclavicle to the upper end of the clavicle.

The pelvic girdle is closely attached along the posterior lower edge of the clavicle. The opposite sides are suturally attached along the median line, and together send back a long stout median spine through the middle of the ventral disk. Toward the anterior end each side bears a large foramen. The ventral fins are attached to the outer edges of the girdle, with their bases directed transversely across the body, or towards each other, rather than being attached, as in other forms, to the posterior ends of the girdle with their bases directed forward.

Vertebral, Rib and Fin Elements.

The abdominal vertebræ number 13, the caudal 19, making with the hypural a total of 33.

The first vertebra is expanded laterally to accommodate the wide occipital condyle. It bears no ribs. The abdominal vertebræ as viewed from below are smooth, without longitudinal ridges or pits, and are much constricted at the middle. On the side each bears a deep pit for the reception of the head of the rib. The last two abdominal vertebræ bear small parapophyses to the under side of the base of which ribs are attached. The base of each abdominal neural arch is expanded laterally over the head of each rib into a horizontal wing-like process, which grows small and disappears posteriorly. Anteriorly each projects in a point at the side of the preceding neural process. These processes are doubtless zygopophyses, though they might be considered parapophyses, as the ribs are expanded upward to their lower surface, were it not for the small processes on the last abdominal vertebræ which, however, prove themselves to be true parapophyses by passing into the hæmapophyses posteriorly. The caudal vertebræ grow more compressed backwards, and exhibit no peculiarities. In *Gobiesox* the vertebral formula is as follows: $14 + 21 + 1 = 36$. The zygopophyses project laterally as in *Caularchus* but are not expanded.

The hypural is symmetrical having a longitudinal incision dividing it into equal parts, each part bearing an equal number of caudal rays. There is no urostyle or other indication of

heterocercy. The spines of one vertebra anterior to the hypural assist in supporting the caudal fin.

Stout ribs are present on all of the abdominal vertebræ except the first one, and are continued back on the anterior caudal vertebræ. They are vertically flattened and grow smaller posteriorly. They project horizontally outward and to the lower surface near the tip of each is a bony ray which curves downward around the abdominal cavity. Gunther[1] describes these as follows: "The epipleurals are not much less developed than the ribs to the extremities of which they are suspended. We might also consider the ribs as long and detached parapophyses, and the epipleurals as the ribs proper." The presence of normal parapophyses on the last abdominal vertebræ prove the latter supposition to be incorrect, and if the distal bones are epipleurals they have changed their usual position on the superior surface near the bases of the ribs to the inferior surface near the tips of the ribs. The only other possibility is that they are extra intermuscular ossifications as occur in some fishes, notably the clupeoid fishes.

No trace of a spinous dorsal remains, and there are no accessory interneural rays in front of the soft dorsal. There are four unbranched ventral rays, and a short flat bone hidden under the skin in front of them representing a ventral spine, the rays are thick and their cross articulations are very close as in the thickened lower pectoral rays of the Cottoid fishes. I do not find in either *Gobiesox* or *Caularchus* the free ventral ray that Dr. Gunther found in *Chorisochismus dentex*.

The interspinal elements are normal in arrangement, and exactly coincide in number with the vertebral spines to which they are attached. No bony baseosts are developed.

Relationships.

The structure of the skeleton gives little help in assigning to the family Gobiesocidæ a definite phylogenetic position, and I find myself no nearer to a solution of the problem than Dr. Gill was when he wrote of these fishes in the "Standard Natural History," (Vol. III., p. 267) as follows: "There are no spines in any of the

[1] Cat. Fish. Brit. Mus., III., p. 493.

fins, and thus it has not even the technical characteristics of the true Acanthopterygians. Doubtless the parentage of the stock is to be looked for in that great suborder; but the divergence of the known forms has been so great that at present it cannot be certainly predicated whereabouts to find it."

I have examined skeletons of Cottoid, Blennioid, and Gobioid fishes with small results. The families Batrachididæ and Callionymidæ offer some slight indications of relationship to the Gobiesocidæ, and the weight of evidence is thrown towards the former family by the young of some or all of them having a ventral sucking disk just behind the base of the pectorals. The family Batrachididæ[1] further resembles the Gobiesocidæ in having the suborbital ring reduced to a small preorbital bone, only very small parapophyses present posteriorly, no myodome, and a single superior pharyngeal present on each side. As opposing the idea of relationship the Batrachididæ have five long actinosts, the posttemporal forms an integral part of the cranium, the palatine is normally joined to the pterygoid, and the mesopterygoid, metapterygoid, alisphenoid, and basibranchials are present.

The family Callynomidæ[2] resembles the Gobiesocidæ in having no mesopterygoid or metapterygoid, thus leaving the symplectic to form part of the anterior border of the cheek bones, in having no myodome or suborbitals, in the ventrals being widely separated, as well as in the general form of the body. The Callionymidæ, however, possess some important and well marked characters not possessed by the Gobiesocidæ, and these probably more than counterbalance the characters held in common. These characters are briefly: a spinous dorsal present; the ethmoid extending back and forming a bony interocular septum; the frontals reduced and occupying little more than the interorbital space; the posttemporal forming an integral part of the cranium; the actinosts all abutting against the hypocoracoid; the hypercoracoid foramen between the coracoid elements cutting an equal notch from each; the palato-quadrate arch normal; three superior pharyngeals present on each side; basibranchials

[1] In this family the following genera were examined: *Batrachoides*, *Opsanus* and *Porichthys*.

[2] *Calleonymus* was the only genus examined.

present; the neuropophyses and hæmopophyses ending each in two spines between which the interspinous elements fit.

SYNOPSIS OF CHARACTERS OF THE FAMILY GOBIESOCIDÆ.

Body broad and depressed in front, covered with smooth naked skin. Premaxillaries protractile. Strong teeth on dentary and premaxillary; none on vomer or palatines; pseudobranchiæ small or wanting. Gill fringes on two and a half or three arches. Occipital condyle partly formed by exoccipitals, which present articulating surfaces entirely lateral to that of the basioccipital. No supraoccipital crest present. Parietals separate or meeting over the surface of the supraoccipital. Myodome absent. No basisphenoid, alisphenoid, opisthotic, mesopterygoid or metapterygoid. Pterygoid reduced. Palatine connected to pterygoid only by a ligament. Preopercle developed backwards in a long triangular process, interposed between, and widely separating the interopercle from the subopercle. No suborbital ring. Basibranchials absent. Fourth ceratobranchial much lengthened. Superior pharyngeals one on each side. Hypohyal single on each side. Six branchiostegal rays. Posttemporal a single ray of bone without a lower limb. Supraclavicle attached just above middle of clavicle. Hypercoracoid foramen through middle of hypercoracoid. Actinosts hour-glass-shaped; two attached to each coracoid element. Postclavicle in two parts; the inferior part supporting the posterior edge of the ventral disk. Spinous dorsal absent. Ventrals each with a concealed spine and four unbranched rays. No airbladder. Parapophyses developed only posteriorly. Zygopophyses produced laterally. A ray of bone attached to each rib extending down around abdominal cavity.

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THE CHROMOSOME COMPLEX OF ORTHOPTERAN SPERMATOCYTES.

C. E. McCLUNG.[1]

A subject of perennial interest is offered by the maturing germ cells, and that appreciation is not lacking is well evidenced by the large annual output of papers devoted to different phases of the question. The task of keeping abreast of this literature has become a considerable one, especially since it is now necessary to take into account the investigations upon hybrid matings and upon unusual or modified methods of fertilization. Despite regret at the increased labor thus brought about, one cannot but rejoice at the enlarged conceptions of chromosome functions which have followed from this union of two apparently different lines of investigation.

It cannot be gainsaid, I think, that as our knowledge increases it becomes more and more evident that in the chromosomes we are dealing with intracellular elements of definite morphological character which are self-perpetuating and which have to do with the development of precise characters in the organism of which they are a part. In my early study of the male germ cells of insects I became convinced of this individuality of the chromosomes, and in all my papers I have emphasized this conception and have brought forward proof in support of my position. It is a pleasure to acknowledge here the material assistance that has been rendered me in this endeavor by the consistent results of my students. Realizing, however, the extensive character of the problem, I have confined the work to a somewhat limited field, but within this have made broad comparative studies. As the work progressed there was indicated the prevalence of a general plan of chromosome structure throughout the tracheate Arthropoda studied and it then became necessary to undertake careful researches within smaller groups, some of which have

[1] I am much indebted for assistance in carrying out work on Orthopteran germ cells to the Carnegie Institution, which made grant No. 16 for this purpose.

already been embodied in papers devoted to the Orthopteran families.

During the course of these investigations it became plain that there were variations from the type of maturation mitoses, and that these afforded facts which seemed to indicate that it might be possible to discover some correlation between individual chromosomes and body characters. It is the purpose of this paper to bring forward some of these facts and to suggest conclusions arising from our thus increased knowledge of the maturation chromosomes. In venturing these deductions I have had in mind their application to the particular forms studied, but at all times have tried to bring them into relation with facts derived from the study of other organisms. This, I believe, is justifiable in the present state of our knowledge, for it is done on the supposition that reproduction is, in the main, the same process throughout the range of organic forms. With the accumulation of observations the conviction is borne in upon us that the maturation mitoses of all organisms are, in general, of one pattern, and that the burden of proof lies upon him who would argue for individual types.

There is always the danger, to be sure, that we have not reached down to the basic facts, but are conceiving more superficial ones to be all important. This is, I imagine, unavoidable in the preliminary stages of an investigation, but it can lead to no harm if the facts in each case have been accurately determined. In the absence, therefore, of definite knowledge of the chromosomes in the germ cells of organisms exhibiting Mendelian characters or mutations we are warranted in supposing them to be of the same general character as the ones known until they are proved different.

The present paper is one of a series in which it is hoped to develop the history of the maturation chromosomes in the Orthoptera. Obviously this is a task of great magnitude and no far-reaching conclusions can be attained until a large series of forms has been studied. Already a not inconsiderable number has been worked over, and each year additions are made. Recently I have been devoting myself again to the family Acrididæ, and in some newly observed forms I have encountered certain peculiari-

ties of chromosome structure which seem to me of considerable importance. Some of these will here be considered.

It will hardly be necessary to enter into any account of the chromosomes in the Acridian family since this subject has already been discussed in a former paper ('00). Familiarity with the spermatogenesis of these Orthoptera will, therefore, be assumed and only such details gone over as are new or, at present, better understood.

The forms upon which the new observations were principally made are three species of the Acridiinæan genus *Hesperotettix*, and a species of *Mermiria*, a Tryxaline. While the facts stated have to do with these forms, they derive their importance from their relation to what appear to be common processes in the groups to which they belong. Much more work is, therefore, involved in the attainment of these results than would appear on first thought, and the basis for theories is correspondingly widened. In addition to the facts gained from the species mentioned, confirmatory evidence on a number of points afforded by some incomplete observations on *Chortophaga viridifasciata*, an Acridian, and *Anabrus*, a Locustid

II. Observations.

1. *Number of Chromosomes in the Family Acrididæ.*

It may be recalled that in previous papers I have not laid much stress upon reported numbers of chromosomes, because of the difficulties involved in securing accurate enumerations. Nevertheless the full importance of this knowledge has not lacked appreciation, and latterly more attention has been directed toward the determination of the number of chromosomes in the different species of Acrididæ. In order to avoid the operation of the personal factor as much as possible, camera lucida drawings were made in considerable numbers from time to time and laid away. Finally the drawings were taken and the counts tabulated. As only the clearest cases were used for drawing, and as every precaution was taken to see that the entire complement of chromosomes for each cell was present, it is thought that the figures are reasonably accurate. Since this is the judgment of one who has at all times been decidedly critical toward the subject of chromosome enumerations, it may lend assurance to the results.

Regarding the enumerations I would say that much confidence is reposed in those of the spermatocyte elements, but that there is less certainty attending the figures derived from the spermatogonia. This is due to the fact that the latter cells are relatively small and the chromosomes very large, so that the elements are not well separated and clear. There is no fusion of the chromosomes in the metaphase, but they are long and sinuous and their limits hard to discern.

In general, then, it was found that for the family the number of chromosomes in the spermatogonia is 23 and in the first spermatocyte 12. Exceptions were found in *Hesperotettix* which has 23 in the spermatogonia and 11 in the first spermatocyte; and in *Mermiria* which has 23 in the spermatogonia and 10 in the first spermatocyte. Careful study revealed the fact that the exceptions are only apparent, and that the reason for the deviation from the family character is due to unusual associations of the spermatogonial chromosomes in the spermatocytes. This is brought about by the action of the accessory chromosome which here gives further evidence of its highly important character. It will be well to outline the history of the chromosomes in these genera separately and then refer to the principles of association involved later.

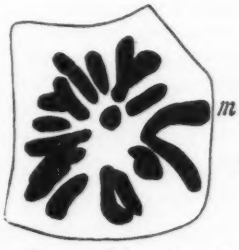

FIG. 1. Polar view of a spermatogonium of *Hesperotettix speciosus* (all the drawings of this genus were made from *speciosus* material) in metaphase, showing a tetrad "*m*." Not all the chromosomes are drawn.

2. *The Chromosomes of Hesperotettix speciosus.*

It is not my intention to discuss the whole group of chromosomes in this species, but merely to give the history of the most strongly marked element. I hope subsequently to make a careful detailed study of the germ cells of the various species of this genus to establish the relations of the entire complement of chromosomes, but at present that is not possible.

If we examine the spermatogonial mitoses of this species we find that the chromosomes are of the usual rod-shape type, but among them there occurs one that is peculiar in being bivalent

instead of univalent. The chromatids composing this multiple chromosome[1] are amongst the largest in the cell and are easily distinguishable. During the anaphase it is seen that the multiple chromosome exhibits the so-called heterotypical form of division, and the figure produced is exactly the same as that resulting from the division of the tetrads in the first spermatocyte. In reality we have here a tetrad that is in every respect the same as those in the succeeding generation. A precocious synapsis of chromosomes in the spermatogonia is responsible for the unusual type of division in this generation of cells.

FIG. 2. The hexad multiple chromosome of *Hesperotettix* in the first spermatocyte prophase. The accessory chromosome "*a.c.*" is homogeneous and the ordinary chromosomes granular, as is usually the case for these elements at this stage.

There can be no mistake about the nature of this multiple chromosome, for the line of fusion between the two chromosomes is clearly marked, and the mantle fibers of the metaphase attach at the center of the U-shaped element. In the anaphase the contrast between this chromosome and the others is very marked, for the daughter chromosomes of the latter ascend to the poles as straight rods with the fibers at the ends, while the former are U-shaped and have the fibers annectent at the center, or where the chromatids are joined together.

The early prophase of the first spermatocyte shows this bivalent element among the others, which have by this time also become bivalent, but it may still be distinguished by its greater length. When the chromosomes commence to shorten and condense the accessory chromosome attaches itself to one end of the large bivalent element, thus forming a trivalent chromosome. As thus constituted the multiple chromosome is much longer than any of the others, and clearly shows the limits of the three parts. A very peculiar condition prevails at this time. As is usually the case in the prophase, the ordinary chromosomes are granular and irregular in outline, while in striking contrast the accessory end of the multiple chromosome is homogeneous with sharply cut boundaries. The trivalent chromosome, or hexad, thus

[1] I shall use the term "multiple chromosome" for all elements containing more than two chromatids.

exhibits a very unusual appearance, because of the heterogeneous condition of its parts. Besides this structural difference, the accessory chromosome is further made noteworthy by the fact that it is shorter than the other thirds of the multiple element.

When the mitotic figure of the first spermatocyte is formed the hexad takes a position parallel to the axis of the spindle with the division between the parts of the original bivalent chromosome in the equatorial plate. As it lies thus it is almost as long as the spindle, but has approximately two thirds of its length on one side of the equatorial plate. This greater portion includes, of course, the accessory chromosome. Since the fibers always attach to the ends of the chromosomes, the accessory becomes bent away from the spindle and lies at a more or less obtuse angle to the rest of the chromosome. The mantle fiber then attaches to the angle thus established. A similar arrangement is mentioned by de Sinéty for the phasmid *Leptynia*.

FIG. 3. Lateral view of the first spermatocyte prophase with a number of thechromosomes drawn. The hexad is shown in profile with the accessory chromosome, "*a.c.*," characteristically bent back at an angle. These, and all other drawings in this paper, were made under a camera lucida and are reproduced here at a magnification of 1,900 diameters.

With the beginning of the anaphase this multiple chromosome separates at the point where the two chromosomes were united in the spermatogonia, so that to each end of the spindle there goes one of the spermatogonial elements, but the accessory chromosome, on the contrary, becomes a member of only one of the daughter nuclei. Thus it happens that in the first spermatocyte where the quantitative division of the other chromosomes occurs this one element is divided qualitatively. In effect this is

also a qualitative division of the accessory chromosome which here exhibits the same behavior as has been described for the Locustids, the difference being that in the case of *Hesperotettix* there is a close association between the accessory and an ordinary tetrad. It would seem that there is here an attempt on the part of the accessory chromosome to establish the usual relationships that are formed between chromosomes at this period of the maturation process. To this extent the element departs from what has been regarded as one of its most characteristic features, that of exclusiveness. As a matter of fact, however, all the later work upon this structure has tended to show similarities to ordinary chromosomes rather than differences from them, and we are not surprised, therefore, to find it uniting itself with another chromosome in the ordinary manner. Being the odd member of the group it is unable to accomplish this step in the usual way and is therefore forced to become a member of a hexad element.

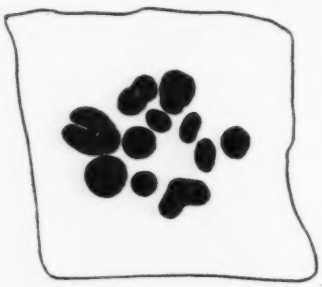

FIG. 4. Polar view of first spermatocyte metaphase, *Hesperotettix*, showing all the chromosomes of the complex.

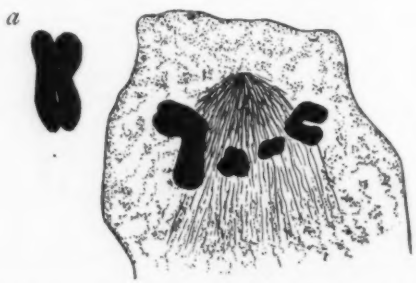

FIG. 5. Lateral view of one pole of first spermatocyte anaphase, *Hesperotettix*, with four chromatic elements drawn. The multiple chromosome at the left consists of the accessory chromosome and one half of the tetrad to which it was united. The relative positions are the same as in Fig. 3. Fig. 5*a* represents such a multiple chromosome *en face*.

Why it should always be the same tetrad that is selected for this association is not clear, and will perhaps become apparent only when we learn the meaning that attaches to the intimate association among the maturation chromosomes.

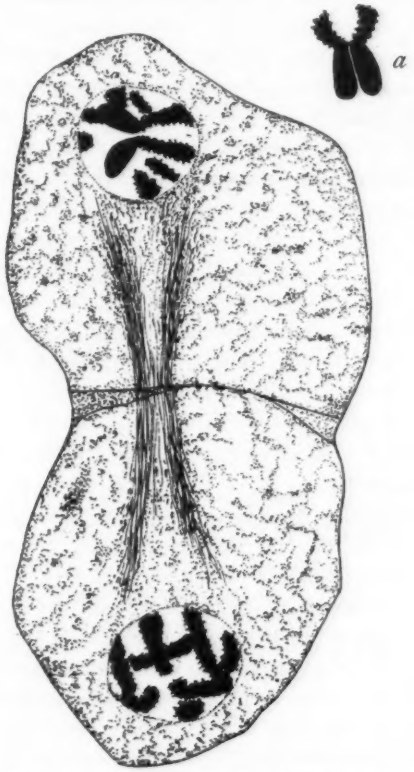

FIG. 6. Telophase of first spermatocyte of *Hesperotettix*. At the upper pole is the multiple chromosome in which the accessory chromosome remains homogeneous while the other element of the tetrad has again become granular. Homologous chromatids are well separated. Fig. 6*a* shows a similar chromosome with the accessory chromatids less divergent.

The close connection thus established by the accessory chromosome persists through the later periods of the maturation process. In the telophase the pair of chromosomes, consisting

of the accessory and the half of the tetrad, very much resembles the ordinary chromatin elements of the prophase, being, as it is, tetrad in character. Here as always, however, the accessory remains homogeneous while the associated chromosome becomes granular like its fellows. With the establishment of the second spermatocyte mitotic figure this oddly constituted tetrad takes its place in the equatorial plate with the mantle fiber attached at the point where the accessory chromosome is joined to the other one. Since the accessory is the shorter the fiber is not inserted at the middle of the chromatin rod but slightly to one side. The normal form and position of the chromosomes of the second spermatocyte in metaphase is that of a split rod pointing radially away from the axis of the spindle with the chromatids placed one above the other in the plane of the spindle axis. While all of the remaining chromosomes of *Hesperotettix* are thus arranged, the accessory chromosome and its mate are bent upon each other so as to present an angle to the spindle. It thus happens that in the anaphase there is apparent a heterotypical division of the multiple chromosome in which the resulting V-shaped loops each have one arm shorter than the other. It is now evident that the chromosome associated with the accessory has taken part in a heterotypical division in the spermatogonia and in the second spermatocyte, and in each case the plane of division corresponds to the original longitudinal cleft of the spireme thread. Because of the early association between the accessory and the tetrad there can be no doubt regarding the planes of division in the hexad.

I have not as yet attempted to work out in detail the specific characters of the *Hesperotettix* chromosomes, although I have that in mind for an early investigation. But even in the preliminary study here presented there have become apparent specific differences in the first spermatocytes which seem to be constant and which I will briefly notice. The description given above is based upon *Hesperotettix speciosus*, a form of larger size than the others and with strongly marked characters. Besides this there are in the same habitat two other species that are somewhat common and which are plainly different from *H. speciosus* but resemble each other in size and general appearance. This close resemblance caused my collectors to confuse the material from

which slides were made, and so I am not sure whether I have *H. pratensis* or *H. viridis*. I am tolerably sure that both are represented, although I have not as yet been able to detect differences enough in the cells to separate the preparations into two groups.

Despite the uncertainty regarding the exact character of the material, it is clear enough that it is not from *H. speciosus*, so that we know that any differences manifest in the cells will be between *speciosus*, definitely identified and described above, and either *viridis* or *pratensis*. From this comparison it is interesting to find

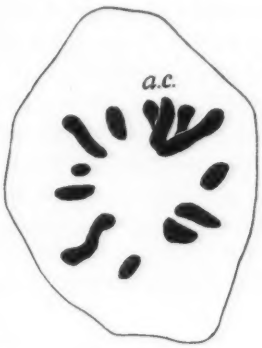

FIG. 7. A polar view of the second spermatocyte of *Hesperotettix* containing the accessory chromosome "*a.c.*" It forms the smaller half of the tetrad. Not all the chromosomes present.

FIG. 8. Fragment of a second spermatocyte of *Hesperotettix* in metaphase showing the tetrad containing the accessory chromosome and two ordinary chromosomes.

that there are constant differences in the germ cells which are correlated with constant differences in body structures. So far I have made no attempt to trace variations beyond those shown by the hexad multiple chromosome. Even here only a start has been made, but it will suffice for my present purpose merely to show that there are differences between species of a genus in germ cell architecture.

First we must notice that there are present in both species multiple chromosomes of the same sort. This must be regarded as a generic character, and for specific differences it will be neces-

sary to look for modifications in size and proportions of parts. In drawings made at a magnification of 2,875 diameters it is found that the accessory chromosome of *H. speciosus* in metaphase measures on an average about 12 mm. while in *H. viridis* (?) it is near 11 mm. This difference is only slight, but if the chromosomes associated with the accessory are measured it will be found that the variation in size is considerable. Thus in *H. speciosus* the average length is in the neighborhood of 20 mm. while in *H. viridis* it is not over 15 mm. Here is a very pronounced specific variation which is probably accompanied by corresponding variations in the other chromosomes. Along with this difference in size of the chromosomes there goes a corresponding variation in the achromatic figure. The spindle in the first spermatocyte of *H. speciosus* is long and slender, while in *H. viridis* (?) it is short and heavy. There are probably many other less obvious differences which I have not discovered in this preliminary survey. I hope in time to present these in detail.

FIG. 9. A similar cell in anaphase. The disproportion between the accessory part of the dyad and the other member is well exhibited.

3. *The Chromosomes of Mermiria sp.*

In the spermatogonia of *Mermiria* there is again found the heterotypical mitosis of chromosomes. The early synapsis of chromosomes, which is the occasion for the unusual form of mitosis, is encountered very frequently in spermatogonia, and I shall have occasion to refer later to instances in certain species where it is very marked. In the prophase of the first spermatocyte of *Mermiria*, there is again found a multiple chromosome which shows the same constitution as the one in *Hesperotettix*. The pronounced difference between the smooth homogeneous accessory chromosome and the rough granular elements of the the tetrad joined to it is strikingly shown in the beautifully clear nuclei of this Tryxaline. So far the case is parallel to that exhibited by *Hesperotettix*, but just before the metaphase a singular and entirely unique association of the hexad multiple chromosome

of the prophase with one of the tetrads occurs. This is brought about by the end to end union of the parts which produces a pentivalent chromosome, or decad. So far as I know no such a chromosome as this has ever before been described.

The metaphase exhibits this strikingly peculiar chromosome extended along the length of the spindle parallel to its axis. The terminal parts are variously placed, sometimes being bent back parallel with the middle portion, sometimes extending out at right angles to it, and again being directed upward at an obtuse angle. Frequently the two terminal elements are differently inclined to the middle portion. In any event the archoplasmic fibers attach, not at the free ends of these chromosomes, but where their other ends join the

FIG. 10. Lateral view of some of the chromosomes in the metaphase of a spermatogonium of *Mermiria*. The multiple chromosome shows its tetrad character well.

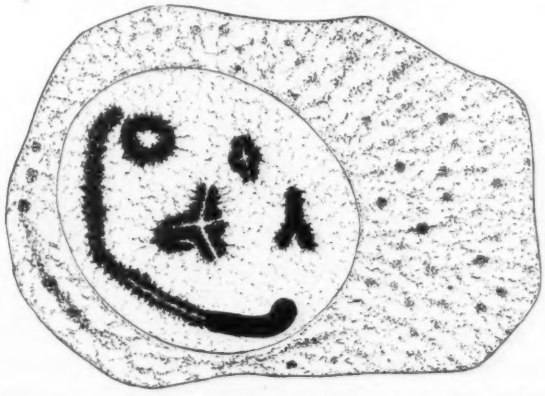

FIG. 11. Prophase of first spermatocyte of *Mermiria* showing particularly the accessory chromosome attached to a tetrad as in *Hesperotettix*. The organization of the cytoplasm is also indicated.

main shaft of the element. One point which I have not yet been able to determine with certainty is the position of the accessory

chromosome in the multiple element. Whether the tetrad that is added to the multiple chromosome attaches to the accessory end of the trivalent element of the prophase, or to the other extremity, I have not made out with certainty. The final result of the divisions would not be different in either case.

Upon the separation of the chromosomes in the metaphase the multiple chromosome is divided so that to one pole there goes a trivalent element and to the other a bivalent one, the difference in valence being due to the presence of the accessory chromosome in one daughter cell. There occurs here an entirely unique separation of chromosomes, for by means of it *entire*

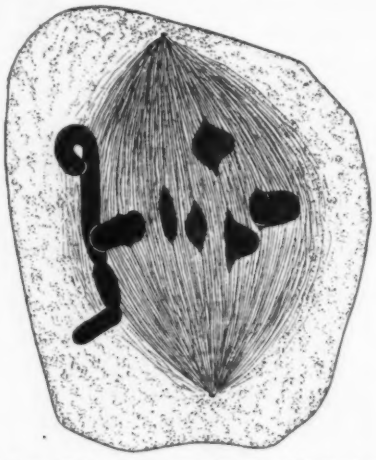

FIG. 12. Metaphase of first spermatocyte in *Mermiria* showing the decad in profile and a number of the tetrads. Observe that the fibers attach at the point of contact between two chromosomes and not at the free ends of the chromosomes. A very large part of the cytoplasm is organized into the spindle and the remainder of it is measurably polarized.

tetrads pass into the second spermatocytes. If these bivalent structures are always produced by the fusion of paternal and maternal chromsomes, then in this case both such pass into one cell. The alternative possibility that they are not homologous chromosomes will be discussed in the latter part of this paper.

When the second spermatocyte mitotic figure is formed it is seen that the accessory has separated from the dyad with which

it was united and has divided longitudinally as usual. But besides the accessory and the ordinary dyads of the second spermatocyte, there is present in each cell a tetrad which was a part of the multiple chromosome and which did not undergo division in the first spermatocyte. This may be found occupying on the second spermatocyte spindle such a position as tetrads usually occupy in the first spermatocyte, and under these circumstances it divides in the customary longitudinal manner. Here again there is a heterotypical mitosis along with the ordinary type, a condition which was also present in the spermatogonia. In both cases there is indisputable evidence that the plane of separation is along the original longitudinal split of the chromatin thread.

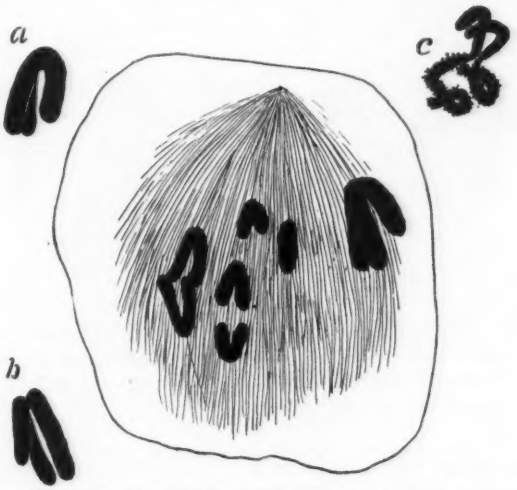

FIG. 13. Early anaphase of first spermatocyte in *Mermiria*. Only one pole of the spindle in the section and a few of the chromosomes. The method of the dyad separation shown in the tetrads. At the right of the spindle is represented a tetrad derived from the decad. Similar elements are shown in "*a*" and "*b*." At "*c*" is drawn the same structure in the telophase, where the homogeneous character of the accessory chromosome is in evidence.

A careful enough study of these cells has not yet been made to determine whether the pair of chromosomes in the second spermatocyte that remain united is the one which reappears in the spermatogonia of the next generation or not. It may not be

possible to ascertain this positively under any circumstances, but I believe that definite enough information could be secured by a comprehensive study. Considerable importance attaches to this as will be shown later.

In the Tryxalines the organization of all the cell contents is complete and precise at the time of the first spermatocyte mitosis. Practically the entire cell takes part in forming the bipolar mitotic figure. It is interesting to observe that the greater portion of

FIG. 14. Portion of a second spermatocyte of *Mermiria* in which the division of the tetrad coming from the decad of the first spermatocyte is shown along with that of the dyads.

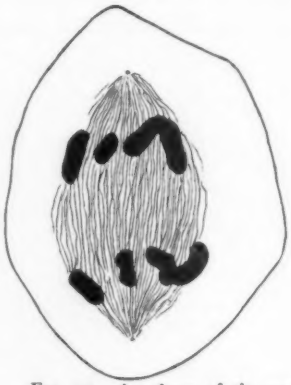

FIG. 15. Anaphase of the second spermatocyte of *Mermiria* showing the separated dyads of the tetrad and halves of ordinary dyads.

the cytoplasmic fibers lie outside of the circle of chromosomes that show in a polar view of the metaphase. At this time the chromosomes are scattered along in the spindle, forming no definite plate, and seem to divide independently and unaided.

This independent action of the chromosomes and the disproportion between them and the cytoplasmic figure would indicate apparently that the formation of the spindle is not to produce a mechanism for the separation of the chromosomes, but is rather for the purpose of securing an accurate division of the cell materials. This assumption is further supported by the observa-

tion that the same chromosomes that are involved in the first spermatocyte mitosis, where the spindle fills the entire cell, in the preceding generation, the spermatogonia, divide around a spindle that is so small as to be practically negligible as a motor apparatus for the movement of the chromatin. The spermatogonial spindle is indeed so diminutive as to be almost invisible among the relatively huge chromosomes that surround it, and so short as to be a fraction of the length of the chromosomes.

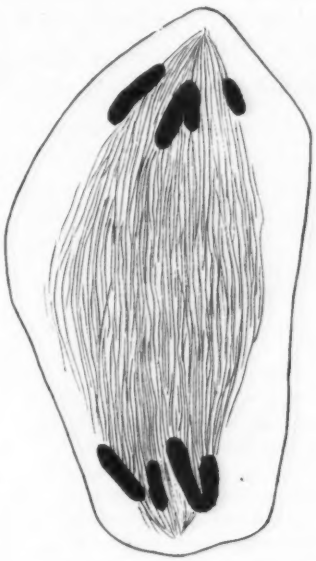

FIG. 16. Late anaphase of second spermatocyte of *Mermiria*. Here are represented later stages in the separation of such elements as are shown in the preceding figure.

If the mitotic figure is purely, or even principally, a mechanical arrangement for the separation of the chromosome halves, then there would be some proportion between the spindles of successive generations which have to do with the same chromosomes. The ability of the chromosomes to execute independent movements is proven by the action of the accesosry in wandering to one pole of the spindle in advance of the other elements, as well as by the long series of movements performed by them in the prophases.

4. *Certain Chromosomes of Chortophaga viridifasciata.*

Besides the instances of unusual chromosome associations mentioned for *Hesperotettix* and *Mermiria* I am able to bring forward others which will serve to show that the conditions present in these forms are not pathological or abnormal. The most pronounced case of synapsis of spermatogonial chromosomes is exhibited by *Chortophaga*, an Œdipodinæ. I observed and photographed cases of this three years ago, but did not attach much

importance to the condition until I recently encountered it in a number of other forms.

The spermatogonia of this genus show numerous pairs of chromosomes when viewed from the pole, in which the evidence of the end to end union of univalent chromosomes is unmistakable. The number of these pairs is not constant, but as many as four have been encountered in one cell. A side view of an

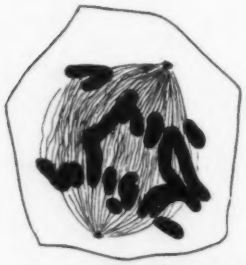

FIG. 17. Lateral view of a spermatogonium of *Chortophaga* in metaphase. The separation of multiple chromosomes along with simple ones is well shown. This is another good illustration of heterotypical divisions in the spermatogonia.

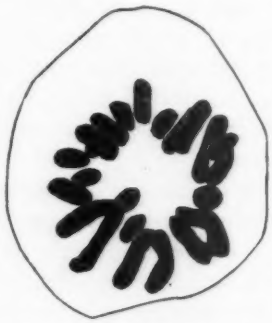

FIG. 18. A polar view of a similar cell in which appear four tetrads. This is an exceptionally good illustration of early synapsis of the chromosomes.

anaphase shows very clear heterotypical divisions among the chromosomes, the remainder of which are divided in the usual way. The subsequent history of these I have not worked out.

5. *Certain Chromosomes of Anabrus.*

During the course of my studies upon Locustid genera I was caused not a little difficulty by an oddly formed chromosome in the spermatocytes of the genus *Anabrus*. At the time I was obliged to confess myself beaten in the endeavor to determine the constitution of this element. That it had to do with the accessory chromosome I was thoroughly convinced, but that it was unusual in some way I was equally assured. Not until I had become familiar with the hexad multiple chromosome of *Hesperotettix* did the matter clear itself up. Having learned the pos-

sibility of an association of the accessory chromosome with one of the bivalent structures of the first spermatocyte I was soon able to see that the peculiar chromosome of *Anabrus* was another such a multiple chromosome as that of *Hesperotettix*. There is this difference between them, however, that in *Hesperotettix* the accessory chromosome is the small, univalent element, while in *Anabrus* it is proportionately very large, causing the tetrad with which it is associated to appear as an appendage at one end.

The multiple chromosome of *Anabrus* is a striking illustration of a fact to which I have often made reference, *i. e.*, to the relation existing between chromosomes and the attached archoplasmic fibers. *In all tetrad elements the fibers are annectent at*

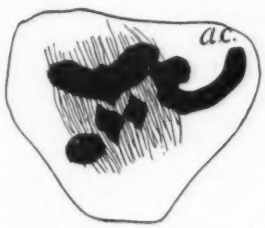

FIG. 19. Oblique view first spermatocyte of *Anabrus* in metaphase in which appears a multiple chromosome consisting of the accessory chromosome "*a.c.*" and a small tetrad, several small tetrads and the one large one.

FIG. 20. Lateral view of first spermatocyte of *Anabrus* in metaphase exhibiting the same structures represented in the preceding figure. Note the attachment of the fibers to the hexad.

the fused ends of the chromosomes, representing the plane of the future reduction division, and never at the free ends. When the accessory is added to a tetrad in *Hesperotettix* the arrangement is disturbed somewhat in order to allow the insertion of one fiber to the point of contact between the accessory chromosome and one member of the tetrad, while the fiber to the other centrosome finds its connection at the free end of the tetrad.

Such a condition obtains also in *Anabrus* resulting in the production of a very singular appearing chromosome. Since the accessory is so very long it seems as if the fibers from both centrosomes attach near one end of the multiple chromosome, but as

a matter of fact one is connected where the accessory joins the tetrad and the other at the opposite end of the tetrad. From the variation in shape of the tetrad portion of the hexad it would appear possible that it has the accessory attached to the side and so divides longitudinally, but this I have not yet determined. Frequently it happens that the free extremity of the long accessory chromosome lies near the pole of the spindle, but in metakinesis it is the other end that moves toward the centrosome. In its passage from the equatorial plate to the pole this end of the chromosome is bent upon the other moiety producing a U-shaped figure. I have not had opportunity to trace the later history of chromosomes thus associated, but have little doubt that they conduct themselves after the manner of similar ones in *Hesperotettix*.

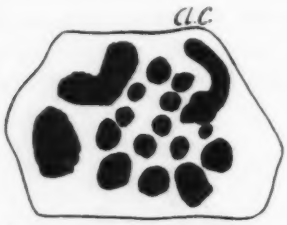

FIG. 21. Polar view of a similar cell with all the chromosomes drawn.

It is thus shown that in three of the Orthopteran families, *i. e.* Acrididæ, Locustidæ, and Phasmidæ (de Sinéty) a hexad multiple chromosome is found in the first spermatocyte of certain species. Doubtless it exists in many others not yet studied and may reasonably be expected to occur in all the families of the order. In the presence of such elements we may also find an explanation of differences in number and behavior of chromosomes in related forms and in such as exhibit peculiar chromosomes not referable to the ordinary types.

SUMMARY OF OBSERVATIONS.

1. An Orthopteran family, the Acrididæ, is characterized by the possession of a definite and fixed number of chromosomes in its germ cells.

2. A genus of this family is marked by the arrangement of this series of chromosomes in a characteristic manner, which results in a precise sequence of divisions for these elements.

3. A species shows the grouping of chromosomes peculiar to the genus, but is distinguished by size differences of chromosomes, spindles and other cell parts.

4. The synapsis of chromosomes in pairs or other combinations does not occur in all species at the same time, nor do all the elements of the cell enter into these relations synchronously. There is accordingly no fixed synaptic phase.

5. Heterotypical divisions are observed in spermatogonia, first spermatocytes, and second spermatocytes, and in each case represent an ordinary longitudinal cleavage of the chromatin thread.

6. In cases of unusual associations of chromosomes the accessory is involved in the formation of the multiple chromosome.

7. Under all conditions the accessory chromosome goes to but one half of the spermatozoa.

8. When pentivalent multiple chromosomes are formed whole tetrads go into the second spermatocytes undivided.

10. In a single mitosis some of the chromosomes may divide qualitatively while the others divide quantitatively. This is due to unusual associations of the elements, and occurs regularly in the same way in all the cells of a generation.

11. Unless chromosomes are associated into multiple elements of higher valence than tetrads they divide longitudinally in the first spermatocyte and transversely in the second.

12. Tetrads divide longitudinally wherever found, either in spermatogonia, first spermatocytes or second spermatocytes. Multiple chromosomes of higher valence than tetrads divide transversely.

Conclusions.

1. *Chromosomes and Somatic Characters.*

Two facts of first importance stand out as a result of these observations. The first is that a definite series of chromosomes accompanies the exhibition of a group of somatic characters that have been utilized by systematists for the inclusion of a number of genera into a family. The second fact is that these genera are characterized by a very precise architecture of their germ cells, whereby the series of chromosomes presents a definite arrangement and association which suffers modification in minor details in the different species of the genus. At the bottom of all, of course, lies the general fact that the chromosomes are morphological elements of the greatest constancy and importance.

Much has been done lately to show that the theory of the individuality of the chromosomes is well founded, but up to this time all that has been attempted has been to prove that in the species there is a constant series of chromosomes that appears in the successive generation of cells in each individual. I think that this has been done beyond reasonable question, in a number of forms and by different means. From the brief comparative study of the Acrididæ that I present here it now appears that not only are the chromosomes constant for the species, but also for the genus and family.

The far-reaching importance of this correlation between somatic characters and germ cell structure is at once apparent, for there is now some hope that it will be possible to establish the relations that exist between body characters and individual chromosomes. This has been the goal toward which I have been working ever since I observed the constancy in the behavior of the accessory chromosome and surmised its connection with the development of the male sexual characters — the first attempt, so far as I know to establish a relation between a particular chromosome and the same group of characters in different species.

The problem is unquestionably one of great complexity and difficulty, but I believe that with careful comparative studies of a great number of genera the rôle of the individual chromosome in development can be ascertained. If it is found possible, as I hope it may be, to apply experimental methods in breeding, etc., to these forms the results may be more quickly brought about. I have already made some tentative experiments, but have as yet accomplished nothing definite. Increased knowledge of the phenomena prevailing in these animals is bound to make the correlation between germ cell architecture and body characters more and more definite in details, and in connection with observations on other forms, will permit the formulation of some general principles that will be applicable to all organisms.

In the present state of our knowledge, however, we can do little more than erect a working hypothesis of a very general character, which doubtless will have to be modified with the apprehension of new groups of facts. It is to be borne in mind that our hypothesis must explain not only the appearance of the

characters in the individual and groups of individuals, but must also offer some explanation of the possible origin of new characters which serve as the basis for new genera and species — or, in other words, of variation. The most we can hope to accomplish by observation, in any event, is to determine the mechanism by which the germ cells operate to produce the body structures — the principle back of this can only be guessed at.

Before undertaking the formulation of any hypothesis it may be well to consider a few questions concerning the nature of body characters and their relation to each other and to the germ cells. The term "character" has a very broad and uncertain meaning. It may be applied to any structure of the organism from a vitally important organ to a trivial marking on the surface of the body. It is very much a question whether the characters belonging to these categories are equally firmly fixed upon the germ plasm, and for our present purpose it is desirable that we know whether there is any relation between the need of a character and its fixity in the chromosome. Again we should know the relations of the characters to each other in development. We shall have to ascertain, for instance, whether an organ or part develops purely as a result of the presence of one particular chromosome or whether the structure owes its initial stages to the action of one chromosome and its later development to the influence of another. It will be necessary to determine to what extent the chromosome acts as an individual and how far it functions as a mutually coöperating element in a complex. These considerations lead finally to the question as to the nature of the individual chromosome at different periods in the development of an organism. Is the chromosome of the just mature germ cell potentially the same as that of the tissue cell in the adult organism, or has the latter become progressively different throughout the ontogeny of the organism? If the second condition obtains then it is necessary to determine the means by which the chromosomes of the germ cells maintain their primitive nature through successive generations while the somatic cells in each acquire their various characteristics.

These considerations force themselves upon one as soon as the attempt is made to establish a relation of any sort between an

individual chromosome and certain characters of the body. Unfortunately we have no answers for these questions and they become, in fact, a part of the general problem.

As has been stated, the ultimate aim of these studies is to determine the relation between individual chromosomes and characters in the body, but this specific knowledge will come last of all and only after the most extensive investigations, so it is needless to say that as yet nothing has been discovered concerning the suspected relationship. What will first be accomplished will be the determination of the nature and behavior of the chromosomes in different species and from these observations some suggestions regarding the mechanism of the chromatin elements in heredity may be advanced. This knowledge may come about by a comparison of the germ cells and body characters in nearly related species, by observing the differences in germ cells of individuals that vary from the type of the species, or finally by experimentally disturbing the normal conditions in the germ cells and observing the effects upon the body. An attack on the problem must begin, then, by a search for differences between the germ cells of related species and between those of related genera, and in the present instance concerns certain forms that show very striking arrangements of the elements.

Upon the theory of the primary importance of the chromosomes in heredity we are forced to assume that the development of the characters in the individual of the genus *Hesperotettix* is the result of a definite composition and arrangement of the chromatic elements in the germ cells. Since definiteness of number, composition, and arrangement of the chromosomes always precedes the exhibition of a certain series of characters in the organism, it must be true that an alteration in either the number, composition, or arrangement of the chromosomes would be followed by the development of a somewhat different series of body characters. As I have previously mentioned, the number of chromosomes does not vary, nor is it possible to detect any difference in the composition of the chromosomes (although of course it occurs), but a characteristic arrangement of the chromosomes distinguishes this genus from others in the family, and so we must conclude that this is genetically connected with the subsequently appearing characters.

Within the well defined genus *Hesperotettix* occur the three clearly marked species, *pratensis*, *speciosus* and *viridis* and the germ cells of these exhibit the same grouping of chromosomes — at least of the ones most clearly distinguishable. Very probably when the complex is more thoroughly studied and understood there may be detected variations that are now obscure. Taking the case of the hexad multiple chromosome as being the clearest element to distinguish in the present state of our knowledge, it is found that in *viridis* (?) the tetrad portion shows a pronounced difference in size from that of the corresponding element in *speciosus*. Along with this are associated differences in the form and size of the spindle, etc. — a condition that may accompany, or be caused by, differences in the chromosomes. These peculiarities are connected with the exhibition of the body characters that are utilized for the purpose of classification. Whether these particular structural peculiarities are of the first importance or otherwise we have no way of determining, and this may delay the progress of our knowledge if there be differences in the power of transmission between characters of different rank in functional importance. We can not, therefore, with our present information, say, for example, that the variations of the structures mentioned are the cause of the difference in the size and proportion of the body in the two species, or whether they have to do with the surface markings on the exoskeleton. But when we know the full history of each chromosome in the complex and can follow its variations in all the species of the genus and can know how it differs from others in nearly related genera, then we may be able to associate a particular chromosome with a definite group of characters.

However, in these minutiæ we shall have to await further and much broader knowledge, and for the present turn our attention to the more general questions that have centered around the larger cytological phenomena in their relation to body characters. It is not my intention to take up the question of the form and sequence of the maturation mitoses, since I intend to treat these matters *in extenso* in a later paper. It will suffice to say here that the enlarged views gained by more recent study have tended only to confirm me in the opinions that I have

previously expressed regarding these matters. Just now I wish to consider the bearing that the peculiar multiple chromosomes of *Hesperotettix*, *Mermiria*, *Chortophaga* and *Anabrus* have upon the general questions of heredity raised by the experimental work of the Mendelian school and by de Vries and his followers.

2. *The Chromosomes and Mendel's Laws.*

The first attempt to establish a correlation between cytological phenomena and the operation of Mendel's laws was made by Sutton in 1903, and was based upon Orthopteran material prepared and studied to a large extent in my laboratory. It was in thorough concordance with the laboratory results and represented the final step in the establishment of the theory of the individuality of the chromosomes and their relation to body characters. Done independently of Montgomery, it yet had as its fundamental idea the belief that half the chromosomes are paternal and half maternal, and that at the time of numerical reduction there is a union of homologous chromosomes in pairs. Montgomery clearly stated this idea of the union of parental chromosomes and should have due credit for it, but it is to Sutton's work that we are indebted for the detailed evidence of this fact, and for its theoretical application to the appearance of alternative characters in normal and hybrid breedings. It is this idea of definite association between chromosomes and characters and the explanation it offers for the purity of the germ cells and the recession of traits that renders the work of Sutton unique and makes it valuable.

There is no necessity for entering into any extended discussion of Sutton's results because, coming opportunely as they did with the renaissance of Mendel's conceptions, they immediately gained attention and are generally well understood. The essential feature of his conclusions is that there are in the somatic and immature germ cells a double series of chromosomes, one derived from the father and one from the mother. In synapsis this double series is united into one by the pairwise fusion of homologous chromosomes which remain in this state until the reduction division when they are separated again into a series which is of neither purely maternal nor paternal origin. This is offered as an explanation of the purity of the germ cells postulated by Mendel.

Sutton's views are formulated from careful comparison of size relations of chromosomes in the different generations of the male germ cells of *Brachystola* and represent the detailed proof of the conception expressed in more general terms by Montgomery.

Regarding the main view that the reduced number of chromosomes in the maturing germ cells is brought about by definite fusion of simple chromosomes into multiple ones of bivalent value, there now appears to be a pretty general agreement, and the older conception of a variety of processes has given way to the conviction that the same plan may be detected in all organisms when sufficiently studied. The final step in this direction is to regard this fusion of elements as a definite one between the same members in different generations of organisms. Such a correlation between germ cell architecture and somatic structure would be purely theoretical were it not for the fact that in the operation of Mendel's law there is an exact parallel between what is accomplished for the chromosomes in the maturation divisions and for particular characters in hybrid matings. While this is not an exact proof it raises the probability almost to a certainty and makes it practically impossible to doubt the accuracy of the hypothesis so far as it applies to purely Mendelian characters.

It is known, however, that many characters do not follow the course of those denominated Mendelian, and it now appears from the observations recorded in this paper that the simplicity of behavior by the chromosomes, assumed always to occur at the time of germ cell maturation, is not universal. The question naturally arises, then, whether the extremely simple explanation offered by Sutton is sufficiently inclusive.

Attention has already been called in previous papers to the probable importance of the prophase of the first spermatocyte in the matter of the hereditary transmission of characters. Upon the theory of the union of homologous chromosomes into pairs by synapsis[1] the nature of this becomes evident, for in the grass-

[1] By synapsis I mean the fusion of simple chromosomes into multiple ones, usually of bivalent value, according to the idea of Moore, who proposed the term. I would suggest that in order to avoid the lamentable confusion that has resulted from the misuse of this designation that a new descriptive word be applied to the condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not. To carry out this idea I shall call this stage the "*synizesis*" of the chromatin.

hopper this pairwise union of chromosomes continues in existence through fully half the life of the individual. If the chromosomes are centers of specific energies it is at this time that they would best be able to influence each other. Montgomery regards this as a phenomenon parallel to what occurs in the conjugation of the lower organisms, and so considers it a process of rejuvenation. This is a very loose term, and according to Montgomery's conception seems to mean nothing more than a stimulus to growth, for it is his conclusion that because of the conjugation of the chromosomes the germ cells immediately proceed to increase in size and attain dimensions in excess of those reached at any other period of their existence.

To me there is a much deeper meaning in this intimate and long continued association of the chromosomes which, as I have recorded for *Chortophaga*, may be established in the early spermatogonia. If there is to be a balancing of forces between homologous chromosomes this would seem to be the place where it must occur. We find, as a matter of fact, that in normal breedings there are produced germ cells that are either entirely pure in regard to certain characters or that develop intermediate results between the two extremes of the parents. The same individuals may show the alternative inheritance of one character and the blended inheritance of another. These phenomena would seem susceptible of a reasonable explanation if we should consider that in the first instance the interaction between the homologous chromosomes was slight, or entirely lacking, while in the second case it was more extensive. Upon the occurrence of the reducing division the linked chromosomes of the first example would separate practically unchanged, while in the other they would pass into the germ cells with different potentialities than they possessed before their synapsis.

If parents of different species were employed we might expect the same results and secure second generations of inbred hybrids that would show pure parental characters or various blends of these. Mosaics might easily be accounted for if we assume that the exchange between the chromosomes has been of such a character as to produce nearly a balance of the alternative characters. Through inequalities of division or by reason of different

environments, now one character would appear, now the other, and so produce the piebald result. Again we might conceive that the difference between two alternatives was so considerable and the disturbance of normal conditions so violent that the whole organization of the chromosomes would be upset with the result that a character distinct from that of either parent would be produced. These various alternatives are realized experimentally and I can see no simpler or more consistant explanation for them than the one I have presented.

3. *Multiple Chromosomes and the Chromosome Complex.*

As I have indicated already, there occur in *Hesperotettix* and *Mermiria* peculiar associations of chromosomes that are consistent from generation to generation of organisms. This can be no accident and undoubtedly has some fundamental meaning. It seems evident that the characters owe their orderly sequence in development to the organization of the germ cell elements and not alone to the mere presence of a particular chromosome. The experimental work of Boveri upon echinoderms and *Ascaris* would indicate the high importance of the individual chromosome, while the observations of Conklin upon the ascidian egg argues strongly for the view that what the chromosomes are able to accomplish in development depends largely upon the materials on which they have to operate — although we must not forget that these materials are probably produced by the action of chromosomes that came from the same original cell as do those of the germ cells.

It seems to me that these apparently somewhat contradictory observations speak unmistakably for the idea of precise organization in the germ cells and for mutual interaction between their parts. This thought comes home to me with particular emphasis after a study of the unusual precision in the arrangement of the cell parts of the spermatocytes of *Mermiria*. The evidence of the pentivalent multiple chromosome is of special importance for it shows that in order to maintain the identity of the chromatin complex from generation to generation there must be an accuracy not only in the division of the chromosomes in maturation but also in their coming together in fertilization. If it were not for

this definiteness of separation and recombination of the chromosomes then there would be all conceivable combinations instead of a definite and constant one such as is always found. An examination of the conditions in this genus cannot fail to put us in possession of some facts that could not well be obtained in any other way. It is necessary to go back to the early first spermatocyte prophase to secure an understanding of the structure of the chromosomes — a point that I have always insisted upon since the beginning of my work upon spermatogenesis. Here it is found that one of the tetrads has become united end to end with the accessory chromosome so that the planes of their longitudinal division are coincident. As is invariably the case in Orthopteran cells the accessory chromosome is univalent, so that there is thus produced a trivalent chromosome like that of *Hesperotettix*. Throughout the length of the chromosome there is a clear longitudinal split which, along the portion contributed by the accessory chromosome, is sharply marked because of the homogeneous character of its elements, while in the remainder of its length it appears somewhat interrupted on account of the granular structure of the tetrad region.

Some time near the dissolution of the nuclear membrane there is joined to this trivalent chromosome, by end to end union, one of the tetrads, thus producing a pentivalent element or decad. This element, we know, has a division running its length and corresponding to the longitudinal split of the chromatic thread, although in its homogeneous condition it is, like that of all the other chromosomes, obscured. In the metaphase of the first spermatocyte, as I have before stated, the separation of the chromosomes takes place in such a way that to one of the daughter cells there goes a tetrad and to the other a tetrad plus the accessory chromosome. For these elements the first maturation mitosis represents a reduction division and one of a very unusual character. At the same time, however, the other chromosomes are dividing longitudinally. Of this there can be no doubt for in the Tryxalines the ring chromosomes are numerous and in *Mermiria* as many as five or six may be found in one equatorial plate. This evidence is unmistakable to one who has a knowledge of their structure gained from a study of their formation in the

prophase, and their position in the equatorial plate, together with their relation to the mantle fibers in the metaphase and anaphase. I cannot stop here to enter into a criticism of the entirely erroneous interpretations of these rings by which de Sinéty proves a double longitudinal division and Montgomery a prereduction.

The probability of the occurrence of equational and reduction divisions in one mitosis is one against which I have previously argued strongly, because whenever reported the cases have lacked the support of thorough observation. The failure of the accessory chromosome to divide in the first spermatocyte mitosis, which is equivalent to a reduction, was the first authentic instance of this complexity, and now we have the case of the multiple chromosome in *Mermiria*, which furnishes further indisputable evidence of the two types of division in one mitosis. In the face of this evidence we must accept the fact and seek an explanation.

This is a matter of secondary importance compared with the question of the probable distribution of the chromosomes to the four spermatozoa arising from each first spermatocyte. It would appear at first glance that from the complexity introduced by the unusual arrangement and distribution of the chromosomes in *Mermiria* there must be a great many possibilities for variation in the chromosome complex, but if we accept the theory of the continuity of the chromosomes and remember that always the same complex appears in each first spermatocyte, then we must conclude that the mechanism of separation, distribution and recombination is a precise one. On the other hand, it is evident that the spermatozoa cannot all be alike, and we have to deal with different categories. This fact has been recognized for some time with regard to the accessory chromosome, but by the unusual combination of parts in the multiple chromosome (decad) of *Mermiria* there comes about an additional differentiation. The precise nature of this I have not been able yet to determine, on account of the absence of some prophase stages in my material. This hiatus I hope to remedy by the acquisition of a larger series of slides, but meanwhile we are sure of some facts and these are sufficient for the present discussion.

Since entire tetrads pass into the second spermatocyte there

are produced four sorts of spermatozoa with regard to the combinations of chromosomes, but two kinds with respect to the presence or absence of certain ones of a multiple. In order that always the same combination of chromosomes (so far as size is concerned, and this appears necessary to satisfy the observations) should appear in the spermatocytes, it would be necessary for eggs with the missing elements to be present and to be fertilized with the proper spermatozoa. The exact nature of this coördinate action cannot now be determined for we are lacking sufficient data, but that it must exist seems apparent from the observation that always the same sized complex reappears in each generation of first spermatocytes. From even our limited knowledge it seems evident that there is great precision and definiteness of organization necessary to bring about in successive generations of similar cells the same grouping of chromosomes. That the entire cell is involved in this organization is, I think, indicated by my observations on the spermatocytes of *Mermiria* where it appears that practically the whole of the cell material is bipolarized and thus accurately divided by mitosis.

The fact that there exists an orderly sequence of concerted movements on the part of the chromosomes suggests many interesting possibilities and offers opportunities for many speculations and theories, but it would seem wise to await the determination of more facts and a careful coöperative study of the species involved before venturing into speculation. Because of the importance attaching to these observations it has seemed desirable to make them known now in the hope that similar phenomena may be observed in other objects. Every additional fact of this sort largely increases the prospect of our attaining an understanding of the mechanism of the hereditary transmission of characters, and if it can be found that a peculiar association of chromosomes is always accompanied by a characteristic arrangement of the body parts then we may begin to see the relation between germ cell architecture and somatic structure.

Among the uncertainties in my mind concerning the behavior of the chromosomes in *Mermiria* is one relating to the nature of the association of the chromosomes into the multiple element of the first spermatocyte. The tetrads seem of the usual type, *i. e.*,

have simple chromosomes of equal size, but when the decad divides it would appear as though there were some heterogeneity present, for in the anaphase one limb of the loop is longer than the other (Fig. 13). This may be due to the formation of a multiple chromosome partly from the accessory chromosome; otherwise it means that the tetrad is not constituted of homologous simple chromosomes. Aside from this there seems to be nothing to contradict the view that the tetrads represent the union of homologous paternal and maternal elements.

4. *Phylogeny of the Chromosomes.*

By the determination of the fact that for the family Acrididæ the number of chromosomes is a constant we gain a new viewpoint for the study of chromosome descent. So long as it was thought that nearly related species were characterized by the possession of different numbers of chromosomes it was impossible to regard the individual chromosomes as very constant structures. We have accordingly such theories as Paulmier's, later endorsed and elaborated by Montgomery, by which certain chromosomes smaller than the others and somewhat different in their behavior in the mitoses are regarded as disappearing from the species and carrying with them the loss of certain characters.

On *a priori* grounds such an occurrence would seem to be very improbable, for the difference between any two species is so slight that the loss of any number of the chromosomes would be entirely disproportionate to the effect. As a matter of fact the difference between two species or two genera consists not so much in the presence or absence of certain characters, but rather in the modification of those possessed in common. If therefore we have a constant relation between a certain group of chromosomes and a certain series of body characters we would expect differences between individuals to come about, not by the loss of chromosomes but by the modification of their structure or relations. It seems to me that the incomplete observations which I am presenting in this paper strongly indicate that this is what takes place.

The Acrididæ are a group of grasshoppers well marked off from the other Orthopteran families by the possession of a series of

characters that are remarkably constant. It is, in fact, sometimes difficult to establish characters of sufficient importance to differentiate subfamilies by their presence or absence. This precision and fixity of bodily organization is accompanied by a corresponding stability of the chromosome complex, which indicates that throughout the family there is a correspondence between individual chromosomes and their products in development. By this I mean that if we can fix upon any particular chromosome by some peculiarity of structure or behavior and associate it with definite body characters we will find that same chromosome in all the species of the family governing the development of the same somatic structures. Individual chromosomes have therefore a continuity of descent in the same way that cells and organisms have.

Comparing the Acrididæ with the Locustidæ it appears that in the spermatogonia there is a difference of ten chromosomes in favor of the latter group. While these two families are well separated it is due more to differences of common characters than to the absence in one of characters possessed by the other. We must conclude, therefore, that the chromatin governing the development of these common characters is present in both families. It is evident, however, from the enumeration of the chromosomes, that it is differently associated in the two groups with the exception of that contained in the accessory chromosome. We cannot for this reason compare chromosomes with chromosomes throughout the whole complex of the two families and it is plain that the differences in structure are due to the differences in composition and association of the chromosomes. This is foreshadowed, or indicated, by the differences of association that prevail between genera, as is suggested by the appearance of multiple chromosomes. These associations may further point the way to an understanding of the differences in number between different families, the smaller one being brought about by the parmanent union of independent chromosomes, rather than by the entire loss of elements.

5. *Chromosomes in Variation.*

A discussion of the relation of chromosomes to one another in different groups leads to an enquiry into the relation of characters

to each other in various individuals. As is well known, between parent and offspring there may be a difference of characters such that if a sufficient series of pairs is taken there may be found an almost continuous seriation. Such variation is appropriately called "continuous." On the other hand it occasionally happens that between parent and offspring there occurs a sudden and sharp rearrangement of body characters, which remains constant. As opposed to the continuous variation this is called "discontinuous." Biologists are more and more inclined to place the origin of variation between parent and offspring in the germ cells and more specifically in the chromatin. Since, however, the types of variation are sharp and distinct there must be corresponding differences in the chromatin. We know so little about the relation this nuclear material bears to the development of characters that it is somewhat hazardous to venture an explanation of the mechanism involved. It seems to me, notwithstanding this, that we may gain some slight insight into the processes by a comparison of the chromosome groups.

Thus in the genus *Hesperotettix* the chromosome complex seems to be a fixed one, and in the different species there occur peculiarities that are constant. We are therefore warranted in assuming that if this uniformity were disturbed it would result in the development of a different series of characters. If, for instance, the hexad of the first spermatocyte in *Hesperotettix* should, for some reason, not be formed at the usual time of the coming together of the tetrad and the accessory chromosome, thus producing a different distribution of the chromosomes into the four spermatocytes, I think we may fairly assume that these cells would not cause the development of the group of characters which we associate with the genus *Hesperotettix*. In such an event we would have a mutation. This, of course, does not explain *why* the mutation occurs, but it accounts for the means by which the result is accomplished. There is in force here, possibly, the same principle that paleontologists have observed to be in operation in groups of animals that are in course of extinction. Here it is noticed that strange and unusual forms appear, and the closing history of the group is marked by the breaking up of the heretofore constant type into numerous and

heterogeneous subtypes. We do not know certainly that this is an occurrence of exactly the same nature as the present day observed mutation, but it is of the same character and is an evidence that stability of the germ plasm is disturbed by irreversible changes which, as I have pointed out, may find expression in different arrangements of the chromosomes.

Opposed to the sudden and pronounced changes in character combinations that mark instances of discontinuous variation stand the common examples of continuous variation, where there is present a graded series of inconstant and fluctuating variations. To my mind the two types of variation suggest different mechanisms of transmission. Discontinuous variation speaks of pronounced and irreversible alterations of the chromosome complex; continuous variation, on the contrary, suggests the idea of small changes in individual chromosomes, due to the impossibility of mechanically producing two exactly equivalent daughter cells. These latter variations are minute and of every conceivable character, but are inconstant and indeterminate. As a basis for the occurrence of these fluctuating changes we have the observed fact of the lack of exact correspondence in the size of chromosomes and other cell parts in the various cells of an organism, and the probability of a difference in the interaction of the chromosomes in synapsis. I can not refrain from again calling attention to the strong evidence that identical twins offer in support of the theory that the characters are fixed by the composition of the germ cells. Since these twins are the product of a single egg it is evident that they must have exactly the same chromosome complex, which, in development, will bring about a very close parallel in the characters produced under their control. No other pair of germ cells from the same two parents will ever produce two individuals that bear the resemblance to each other that identical twins do, because, by the laws of chance, it is improbable that any two pass through synapsis and the maturation mitoses and emerge from them with their chromosomes constituted in exactly the same way. Ordinary twins, coming from different eggs, are no more alike than children of successive births although they are developed under practically identical conditions, thus removing the possibility of environ-

mental differences. Therefore it appears that the fluctuating variations which occur in a series of organisms comprising a species are the results of minor differences in the chromosomes of the germ cells, due to the impossibility of exactly duplicating the conditions of union and separation of the paternal and maternal chromosomes in maturation.

Mutations on the other hand follow some unusual disturbances in the relations of chromosomes to each other, brought about by some change in the germ plasm whose cause we cannot now determine. So much, I believe, we may conclude from our present knowledge of the germ cells and their behavior in maturation and fertilization. As our detailed knowledge of the chromosomes increases we may be enabled to speak more definitely and certainly, but further information we must have and it is earnestly to be hoped that the chromosome groups of many species of these Orthoptera may be carefully and exhaustively studied in connection with observations upon body variation so that correlation between the two may be established.

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June 21, 1905.

NEW TERMS EMPLOYED.

Definitions and Classifications of Chromosomes.

Chromosomes are chromatin elements acting as unit structures during mitosis.

Chromosomes are of two general classes.

1. Simple — containing two chromatids in metaphase.
2. Multiple — containing more than two chromatids in metaphase and formed by the union of simple chromosomes.
   - (*a*) Tetrads, containing four chromatids.
   - (*b*) Hexads, containing six chromatids.
   - (*c*) Octads, containing eight chromatids (not yet observed).
   - (*d*) Decads, containing ten chromatids.

A chromatid is a half of a simple chromosome.

"Synizesis" — the unilateral or central contraction of the chromatin in the nucleus during the prophase of the first spermatocyte. A term proposed to avoid the misuse of the word "synapsis."

PAPERS CITED.

Boveri, Th.

'02 Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Vehr. d. Phys. Med. Ges. zu Wurzburg, 35.

'04 Protoplasmadifferenzierung als auslösender Faktor für Kernverschiedenheit. Sitzungb. d. Phys.-med. Ges. zu Wurzburg.

Conklin, E. G.

'05 Organ-forming Substances in the Eggs of Ascidians. Biol. Bull., 8, No. 1.

'05 Mosaic Development of Ascidian Eggs. Jour. Exp. Zoöl., 2, No. 2.

McClung, C. E.

'00 The Spermatocyte Divisions of the Acrididæ. Kan. Univ. Quart., 9, No. 1.

'02a The Accessory Chromosome — Sex Determinant? Biol. Bull. 3, Nos. 1 and 2.

'02b The Spermatocyte Divisions of the Locustidæ. Kan. Univ. Sci. Bull., 1, No. 8.

Montgomery, T. H., Jr.

'01 A Study of the Germ Cells in Metazoa. Trans. Am. Phil. Soc., 20.

'05 The Spermatogenesis of Syrbula and Lycosa, etc. Proceed. Acad. Nat. Sci. of Philadelphia.

Paulmier, F. C.

'99 The Spermatogenesis of Anasa tristis. Journ. Morph., 15, supplement.

de Sinéty, R.

'01 Recherches sur la Biologie et l'Anatomie des Phasmes. La Cellule.

Sutton, W. S.

'02 On the Morphology of the Chromosome Group in Brachystola magna. Biol. Bull., 4, No. 1.

'03 The Chromosomes in Heredity. Biol. Bull., 4, No. 5.